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(54) Title: IMMUNOGENIC COMPOSITIONS FOR STREPTOCOCCUS PYOGENES

(57) Abstract: The invention includes a GAS antigen, GAS 40, which is particularly suitable for use either alone or in combinations with additional GAS antigens, such as GAS 117, GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS 384, GAS 202, and GAS 057.

IMMUNOGENIC COMPOSITIONS FOR *STREPTOCOCCUS PYOGENES*

All documents cited herein are incorporated by reference in their entirety.

CROSS REFERENCE TO RELATED APPLICATIONS, FROM WHICH PRIORITY IS CLAIMED

This application incorporates by reference in their entirety U.S. provisional patent application No. 60/491,822, filed on July 31, 2003, and U.S. provisional patent application No. 60/541,565, filed on February 3, 2004.

FIELD OF THE INVENTION

This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens derived from *Streptococcus pyogenes* and their use in immunisation. All documents cited herein are incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

Group A streptococcus ("GAS", *S.pyogenes*) is a frequent human pathogen, estimated to be present in between 5-15% of normal individuals without signs of disease. When host defences are compromised, or when the organism is able to exert its virulence, or when it is introduced to vulnerable tissues or hosts, however, an acute infection occurs. Related diseases include puerperal fever, scarlet fever, erysipelas, pharyngitis, impetigo, necrotising fasciitis, myositis and streptococcal toxic shock syndrome.

GAS is a gram positive, non-sporeforming coccus shaped bacteria that typically occurs in chains or in pairs of cells. Although *S.pyogenes* may be treated using antibiotics, a prophylactic vaccine to prevent the onset of disease is desired. Efforts to develop such a vaccine have been ongoing for many decades. While various GAS vaccine approaches have been suggested and some approaches are currently in clinical trials, to date, there are no GAS vaccines available to the public.

It is an object of the invention to provide further and improved compositions for providing immunity against GAS disease and/or infection. The compositions preferably include GAS 40, a GAS virulence factor identified by Applicants, which is particularly suitable for use in vaccines. In addition, the compositions are based on a combination of two or more (*e.g.* three or more) GAS antigens.

SUMMARY OF THE INVENTION

Applicants have discovered a group of thirty GAS antigens that are particularly suitable for immunisation purposes, particularly when used in combinations. In addition, Applicants have identified a GAS antigen (GAS 40) which is particularly immunogenic used either alone or in combinations with additional GAS antigens.

The invention therefore provides an immunogenic composition comprising GAS 40 (including fragments thereof or a polypeptide having sequence identity thereto). A preferred fragment of GAS 40 comprises one or more coiled-coil regions. The invention further includes an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to ten GAS antigens, wherein said combination includes GAS 40 or a fragment thereof or a polypeptide having sequence identity thereto. Preferably, the combination consists of three, four, five, six, or seven GAS antigens. Still more preferably, the combination consists of three, four, or five GAS antigens.

The invention also provides an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of a first antigen group, said first antigen group consisting of: GAS 117, GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS 384, GAS 202, and GAS 057. These antigens are referred to herein as the 'first antigen group'. Preferably, the combination of GAS antigens consists of three, four, five, six, seven, eight, nine, or ten GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens consists of three, four, or five GAS antigens selected from the first antigen group.

GAS 39, GAS 40, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511 are particularly preferred GAS antigens. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination includes GAS 40.

Representative examples of some of these antigen combinations are discussed below.

The combination of GAS antigens may consist of three GAS antigens selected from the first antigen group. Accordingly, in one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and a third GAS antigen selected from the first antigen group. Preferred combinations include GAS 40, GAS 117 and a third GAS antigen selected from the group consisting of GAS 39, GAS 57, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.

In another embodiment, the combination of GAS antigens consists of GAS 40 and two additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40 and two GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511. In another embodiment, the combination of GAS antigens consists of GAS 117 and two additional GAS antigens selected from the first antigen group.

The combination of GAS antigens may consist of four GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and two additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40, GAS 117, and two GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.

In another embodiment, the combination of GAS antigens consists of GAS 40 and three additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40 and three additional GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511. In one embodiment, the combination of GAS antigens consists of GAS 117 and three additional antigens selected from the first antigen group.

The combination of GAS antigens may consist of five GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and three additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40, GAS 117 and three additional GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.

In another embodiment, the combination of GAS antigens consists of GAS 40 and four additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40 and four additional GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511. In one embodiment, the combination of GAS antigens consists of
5 GAS 117 and four additional GAS antigens selected from the first antigen group.

The combination of GAS antigens may consist of eight GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and six additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40 and seven additional GAS antigens selected from the first antigen group. In
10 one embodiment, the combination of GAS antigens consists of GAS 117 and seven additional GAS antigens selected from the first antigen group.

The combination of GAS antigens may consist of ten GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and eight additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS
15 antigens consists of GAS 40 and nine additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 117 and nine additional GAS antigens selected from the first antigen group.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 identifies a leader peptide sequence, two coiled-coil sequences, a leucine zipper
20 sequence and a transmembrane sequence within a GAS 40 amino acid sequence.

FIGURE 2 depicts a schematic of GAS 40 identifying a leader peptide sequence, two coiled-coil sequences, a leucine zipper sequence and a transmembrane sequence, as well as coiled-coil regions of GAS 40 which have low level homology with other Streptococcal proteins of known or predicted function.

FIGURE 3 includes the BLAST alignment analysis of identified coiled-coil regions of GAS 40 with
25 other Streptococcus bacteria.

FIGURE 4 provides predicted secondary structure for an amino acid sequence of GAS 40.

FIGURE 5 schematically depicts the location of GAS 40 within the GAS genome. It also includes comparison schematic depicting a GAS mutant with GAS 40 deleted. Further details on these schematics demonstrate the likelihood that GAS 40 was acquired by horizontal transfer through a transposon factor.
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FIGURE 6 provides comparison FACS analysis depicting the surface exposure of GAS 40 in a wild type strain (and no surface exposure in the GAS 40 deletion mutant).

FIGURE 7 presents opsonophagocytosis data for GAS 40 (in various expression constructs).

FIGURE 8 presents immunization and challenge data for several GAS antigens of the invention.

DETAILED DESCRIPTION OF THE INVENTION

35 As discussed above, the invention provides compositions comprising a combination of GAS antigens, wherein the combinations can be selected from groups of antigens which Applicants have identified as being particularly suitable for immunization purposes, particularly when used in combination. In particular, the invention includes compositions comprising GAS 40.

GAS 40 and the other GAS antigens of the first antigen group are described in more detail below.

Genomic sequences of at least three GAS strains are publicly available. The genomic sequence of an M1 GAS strain is reported at Ferretti et al, PNAS (2001) 98(8):4658 – 4663. The genomic sequence of an M3 GAS strain is reported at Beres et al., PNAS (2002) 99(15):10078 – 10083. The genomic sequence of an M18 GAS strain is reported at Smooet et al., PNAS (2002) 99(7):4668 – 4673. Preferably, the GAS antigens of the invention comprise polynucleotide or amino acid sequence of an M1, M3 or M18 GAS strains. More preferably, the GAS antigens of the invention comprise a polynucleotide or amino acid sequence of an M1 strain.

As there will be variance among the identified GAS antigens between GAS M types and GAS strain isolates, references to the GAS amino acid or polynucleotide sequences of the invention preferably include amino acid or polynucleotide sequences having sequence identity thereto. Preferred amino acid or polynucleotide sequences have 50% or more sequence identity (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more). Similarly, references to the GAS amino acid or polynucleotide sequences of the invention preferably include fragments of those sequences, (i.e., fragments which retain or encode for the immunological properties of the GAS antigen). Preferred amino acid fragments include at least *n* consecutive amino acids, wherein *n* is 7 or more (e.g., 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred polynucleotide fragments include at least *n* consecutive polynucleotides, wherein *n* is 12 or more (e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). In one embodiment, the amino acid or polynucleotide fragments of the invention are not identical to amino acid or polynucleotide sequences from other (non-GAS) bacteria (e.g., the fragments are not identical to sequences in other *Streptococcus* bacteria).

(1) GAS 40

GAS 40 corresponds to M1 GenBank accession numbers GI:13621545 and GI:15674449, to M3 GenBank accession number GI: 21909733, to M18 GenBank accession number GI:19745402, and is also referred to as 'Spy0269' (M1), 'SpyM3_0197' (M3), 'SpyM18_0256' (M18) and 'prgA'. GAS 40 has also been identified as a putative surface exclusion protein. Amino acid and polynucleotide sequences of GAS 40 from an M1 strain are set forth below and in the sequence listing as SEQ ID NOS: 1 and 2.

SEQ ID NO: 1

MDLEQTKPNQVKQKIALTSTIALLSASVGVSHQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVEKTLSSQKAE
LTELATALTKTTAEINHLKEQQDNEQKAL TSAQEIYTNTLASSEETLLAQGAHQRELTATETELHNAQADQHSKETALS
EQKASISAETTRAQDLVEQVKTSEQNIAKLNAMI SNPDAITKAAQTANDNTKALSSELEKAKADLENQKAKVKKQLTEEL
AAQKAALAEKEAEL SRLKSSAPSTQDSIVGNNTMKAPQGYPLEELKKLEASGYIGSASYN NY YKEHADQI IAKASPGNQ
NQQYQDI PADRNRFVDPDNLTP EVQNELAQFAAHMINSVRRQLGLPPVTVTAGSQEFARLLSTSYKKTHGNTRPSFVYGQP
GVSGHYGVGPHDKTIIEDSAGASGLIRNDDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYGHAINFLRVD
KHNP NAPVYLG FSTSNVGLSNEHFVMPESNIANHRFNKTP I KAVGSTKDYAQRVGTVSDTIAAIKGVSSLENRLSAI
HQEADIMAAQAKVSQ LQGLASTLQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLASLKAALHQTE
ALAEQAAARVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTTSSLLNAQEALALQAKQSSLEATIATTEH
QLTLLKTLANEKEYRHLDEDIATVPDLQVAPPLTGKPLSYSKIDTTPLVQEMVKETKQLLEASARLAAENTSLVAEALV
GQTSEMVASNAIVSKITSSITQPSKTSYSGSGSSTTSNLSIDVDESTQRALKAGVVMLAAVGLTGFRFRKESK

SEQ ID NO: 2

ATGGACTTAGAACAAACGAAGCCAAACCAAGTTAAGCAGAAAATTGCTTTAACCTCAACAATTGCTTTATTGAGTGCCAG
TGTAGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGGCGAGTAATACTCACGACGATAGTT
TACCAAAACCAGAAACAATTCAAGAGGCAAAGGCAACTATGATGCAGTTGAAAAACTCTCAGTCAACAAAAAGCAGAA

CTGACAGAGCTTGCTACCGCTCTGACAAAACTACTGCTGAAATCAACCCTTAAAAAGAGCAGCAAGATAATGAACAAAA
 AGCTTTAACCTCTGCACAAGAAATTTACTACTAATACTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAAC
 ATCAAAGAGAGTTAACAGCTACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACTGCATTGTCA
 5 GAACAAAAAGCTAGCATTTCAGCAGAAACTACTCGAGCTCAAGATTTAGTGGAACAAGTCAAACGCTCTGAACAAAATAT
 TGCTAAGCTCAATGCTATGATTAGCAATCCTGATGCTATCACTAAAGCAGCTCAAACGGCTAATGAATAATACAAAAGCAT
 TAAGCTCAGAATTGGAGAAGGCTAAAAGCTGACTTAGAAAATCAAAAAGCTAAAAGTTAAAAAGCAATTGACTGAAGAGTTG
 GCAGCTCAGAAAAGCTGCTCTAGCAGAAAAAGAGGCAGAAGACTTAGTCTGCTTAAATCCTCAGCTCCGCTCTACTCAAGATAG
 CATTGTGGGTAATAATACCATGAAAGCACCGGACAGCTATCCTCTTGAAGAAGCTTAAAAAATTAGAAGCTAGTGGTTATA
 10 TTGGATCAGCTAGTTACAATAATTATTACAAAAGAGCATGCAGATCAAATTTATGCCAAAGCTAGTCCAGGTAATCAATTA
 AATCAATAACCAAGATATTCCAGCAGATCGTAATCGCTTTGTTGATCCCATAATTTGACACCAGAAAGTGCAAAAATGAGCT
 AGCGCAGTTTGAGCTCACATGATTAATAGTGTAAAGAAGACAATTAGGTCTACCACCAGTTACTGTTACAGCAGGATCAC
 AAGAATTTGCAAGATTACTTAGTACCAGCTATAAGAAAACCTCATGGTAATACAAGACCATCATTTGTCTACGGACAGCCA
 GGGGTATCAGGGCATTATGGTGTGGGCCCTCATGATAAAACTATTATTGAAGACTCTGCCGGAGCGTCAGGGCTCATTCG
 AAATGATGATAACATGTACGAGAAATATCGGTGCTTTTAAACGATGTGCATACTGTGAATGGTATTAAACGTGGTATTTATG
 15 ACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAAATACATACGGCCATGCTATTAACTTTTTACGTGTAGAT
 AAACATAACCTAATGCGCCTGTTTACCTTGGATTTTCAACCAGCAATGTAGGATCTTTGAATGAACACTTTGTAAATGTT
 TCCAGAGTCTAACATTGCTAACCATCAACGCTTTAATAAGACCCCTATAAAAAGCCGTTGGAAGTACAAAAGATTATGCC
 AAAGAGTAGGCACGTATCTGATACTATTGCAGCGATCAAAGGAAAAGTAAAGCTCATTAGAAAATCGTTTGTGCGCTATT
 CATCAAGAAAGCTGATATTATGGCAGCCCAAGCTAAAGTAAGTCAACTTCAAGGTAAATTAGCAAGCACACTTAAGCAGTC
 20 AGACAGCTTAAATCTCCAAGTGAGACAATTAATGATACTAAAGGTTCTTTGAGAACAGAATTACTAGCAGCTAAAGCAA
 AACAAGCACAACTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTGAAAGCCGCACTGCACCAGACAGAA
 GCCTTAGCAGAGCAAGCCGCAGCCAGAGTGACAGCACTGGTGGCTAAAAAAGCTCATTGCAATACTAAGGGACTTTAA
 ATTTGAATCCTAACCGCTTCAAGTGATACGTGAGCGCATTGATAATACTAAGCAAGATTTGGCTAAAACCTACCTCATCTT
 25 TGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCTAAACAAAGCAGTCTAGAAGCTACTATTGCTACCACAGAACAC
 CAGTTGACTTTGCTTAAAACCTTAGCTAACGAAAAGGAATATCGCCACTTAGACGAAGATATAGCTACTGTGCCGATTT
 GCAAGTAGCTCCACCTCTTACGGGCGTAAAACCGCTATCATATAGTAAAGATAGATACTACTCCGC TTGTTCAAGAAATGG
 TTAAAGAAACGAAACAACTATTAGAAGCTTTCAGCAAGATTAGCTGCTGAAAATACAAGTCTTGTA GCAGAAGCGCTTGTT
 GGCCAAAACCTCTGAAATGGTAGCAAGTAATGCCATTGTGTCTAAAAATCACATCTTCGATTACTCAGCCCTCATCTAAGAC
 ATCTTATGGCTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTGATGAAAGTACTCAAA GAGCTCTTAAAGCAG
 30 GAGTCGTCATGTTGGCAGCTGTCGGCCTCACAGGATTTAGGTTCCGTAAGGAATCTAAGTGA

Preferred GAS 40 proteins for use with the invention comprise an amino acid sequence: (a) having
 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
 98%, 99%, 99.5% or more) to SEQ ID NO: 1; and/or (b) which is a fragment of at least *n* consecutive amino
 35 acids of SEQ ID NO: 1, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80,
 90, 100, 150, 200, 250 or more). These GAS 40 proteins include variants (*e.g.* allelic variants, homologs,
 orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 1. Preferred fragments of (b) comprise an epitope from
 SEQ ID NO: 1. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15,
 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20,
 40 25 or more) from the N-terminus of SEQ ID NO: 1.

For example, in one embodiment, the underlined amino acid sequence at the N-terminus (leader
 sequence) of SEQ ID NO: 1 is removed. (The amino acid and polynucleotide sequences for this N terminal
 leader sequence are listed in the sequence listing as SEQ ID NOS: 3 and 4. The amino acid and
 polynucleotide sequences for the remaining GAS 40 fragment are listed in the sequence listing as SEQ ID
 45 NOS: 5 and 6.)

As another example, in one embodiment, the underlined amino acid sequence at the C-terminus
 (transmembrane region) of SEQ ID NO: 1 is removed. (The amino acid and polynucleotide sequences for
 this transmembrane region are listed in the sequence listing as SEQ ID NOS: 7 and 8. The amino acid and
 polynucleotide sequences for the remaining GAS 40 fragment are listed in the sequence listing as SEQ ID
 50 NOS: 9 and 10).

Other fragments may omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

Further illustration of domains within GAS 40 is shown in FIGURES 1 and 2. As shown in these figures, an amino acid sequence for GAS 40 (SEQ ID NO: 1) contains a leader peptide sequence within amino acids 1 – 26 (for example SEQ ID NO: 3), a first coiled-coil region within amino acids 58 – 261 (SEQ ID NO: 12), a second coiled coil region generally within amino acids 556 – 733 (SEQ ID NO: 13), a leucine zipper region within amino acids 673 – 701 (SEQ ID NO: 14) and a transmembrane region within amino acids 855 – 866 (SEQ ID NO: 11). Figure 1 depicts these regions within an amino acid sequence for GAS 40, while Figure 2 depicts these regions schematically along the length of the GAS 40 protein.

The coiled-coil regions identified within GAS 40 are likely to form alpha helical coils. These structures are frequently involved in oligomerization interactions, for example between different regions of the protein or between regions of two separate proteins. The leucine zipper motif within the second coiled-coil region contains a series of leucine (or isoleucine) amino acid residues, spaced in such a way as to allow the protein to form a specialized oligomerization interaction between two alpha helices. In a leucine zipper motif, preferably, there are six amino acid residues interspaced between the repeating leucine residues. In a leucine zipper oligomeric structure, the alpha helices are thought to be held together by hydrophobic interactions between leucine residues, which are located on one side of each helix. Leucine zipper motifs are frequently involved in dimerization interactions. The location of the leucine zipper motif within the coiled-coil region further indicates the likelihood that this region of the GAS 40 protein is involved in an oligomerization interaction.

FIGURE 2 also illustrates that there is low level homology between some of the identified regions of GAS 40 and other Streptococcal proteins with known or predicted two dimensional structures or surface localization. Such low level homology may indicate a similar secondary structures or even function. For example, amino acids 33 to 324 of GAS 40, including the first coiled-coil region, has approximately 22% sequence identity to a region (amino acids 112 to 392) of a protein from *Streptococcus gordonii* called streptococcal surface protein A (“SpA”) precursor (Genbank reference GI 25990270, SEQ ID NO: 15). This protein is thought to be a surface protein adhesion, involved in the adhesion of that Streptococcus with mammalian host cell membranes. The *S. gordonii* SpA is a member of streptococcal antigen I/II family of protein adhesions and recognizes salivary agglutinin glycoprotein (gp-340) and type I collagen. Amino acids 33 to 258 of GAS 40 also show low level sequence identify (23%) with another *S. gordonii* protein, Streptococcal surface protein B precursor (Genbank reference GI 25055226, SEQ ID NO: 16).

A similar region of GAS 40 which also overlaps with the first coiled-coil region (amino acids 43 – 238) demonstrates about 23% sequence identity to a region (amino acids 43 – 238) of a protein from *Streptococcus pneumoniae* called surface protein pspA precursor (Genbank reference GI 282335, SEQ ID NO: 17). The aminoterminal domain of pspA is thought to be essential for full pneumococcal virulence, and monoclonal antibodies raised against it protect mice against pneumococcal infections. The pspA domain has a monomeric form with an axial shape ratio of

approximately 1:12, typical of fibrous proteins. Sequence analyses indicates an alpha-helical coiled-coil structure for this monomeric molecule with only few loop-type breaks in helicity.

The second coiled-coil region of GAS 40 has about 46% sequence identity to a region (amino acids 509 – 717) of a protein from *Streptococcus equi* called immunoreactive protein Se89.9 (Genbank reference GI 2330384, SEQ ID NO: 18) (the full length sequence for Se89.9 is also available at <http://pedant.gsf.de>). This *Streptococcus equi* protein is predicted to be surface exposed. BLAST alignment of each of these Streptococcal sequences with GAS 40 is presented in Figure 3.

Further illustration of the two dimensional structure of GAS 40 is shown in Figure 4. First, Figure 4(a) presents predicted secondary structure analysis aligned against the amino acid sequence for GAS 40. The predicted alpha helical regions in Figure 4 generally correspond to the previously noted coiled-coil regions. In Figure 4(b), PairCoil prediction is used to predict the location of putative coiled-coils. Here, two coil regions are identified, generally corresponding to the first and second coiled coil regions. Figure 4(c) highlights the leucine zipper region and illustrates the regularly repeating leucine (or isoleucine) amino acid residues which are likely to participate in the leucine zipper.

Accordingly, the first coiled-coil region of GAS 40 comprises an amino acid sequence of at least ten (e.g., at least 10, 13, 15, 18, 20, 25, 30, 35, 40, 50, 70, 90, 100 or more) consecutive amino acid residues, selected from the N-terminal half of a full length GAS 40 sequence, and predicted to form an alpha-helical complex based on the functional characteristics of the amino acid residues in the sequence. SEQ ID NO: 12 is a preferred first coiled-coil region of GAS 40.

The second coiled-coil region of GAS 40 comprises an amino acid sequence of at least ten (e.g., at least 10, 13, 15, 18, 20, 25, 30, 35, 40, 50, 70, 90, 100 or more) consecutive amino acid residues, selected from the C-terminal half of a full length GAS 40 sequence, and predicted to form an alpha-helical complex based on the functional characteristics of the amino acid residues in the sequence. The second coiled-coil region preferably includes a leucine zipper motif. SEQ ID NO: 13 is a preferred second coiled-coil region of GAS 40.

The coiled-coil regions of GAS 40 are likely to be involved in the formation of oligomers such as dimers or trimers. Such oligomers could be homomers (containing two or more GAS 40 proteins oligomerized together) or heteromers (containing one or more additional GAS proteins oligomerized with GAS 40). Alternatively, the first and second coiled-coil regions may be interacting together within the GAS 40 protein to form oligomeric reactions between the first and second coiled-coil regions.

Accordingly, in one embodiment, the compositions of the invention include a GAS 40 antigen in the form of an oligomer. The oligomer may comprise two more GAS 40 antigens or fragments thereof, or it may comprise GAS 40 or a fragment thereof oligomerized to a second GAS antigen. Preferred GAS 40 fragments comprise an amino acid sequence selected from the group consisting of the first coiled-coil region

and the second coiled-coil region. Such preferred GAS 40 fragments may be used alone or in the combinations of the invention.

The GAS polynucleotides and amino acid sequences of the invention may be manipulated to facilitate or optimise recombinant expression. For example, the N-terminal leader sequence may be replaced with a sequence encoding for a tag protein such as polyhistidine (“HIS”) or glutathione S-transferase (“GST”). Such tag proteins may be used to facilitate purification, detection and stability of the expressed protein. Variations of such modifications for GAS 40 are discussed below. Such modifications can be applied to any of the GAS proteins of the invention.

An example of a GAS 40 sequence with both a GST and a HIS tag is denoted herein as “GST 40 HIS”. This construct includes a GAS 40 sequence where the leader sequence is removed, a GST tag coding sequence is added to the N-terminus, and a HIS tag coding sequence is added to the C-terminus (using, for example, a pGEXNNH vector with NdeI and NotI restriction sites). Polynucleotide and amino acid sequences for the fused region of the GST tag, the GAS 40 sequence and the C-terminus HIS tag of GST 40 HIS are shown in SEQ ID NOS: 19 and 20.

Alternatively, a single tag sequence may be used. An example of a GAS 40 sequence with just a HIS tag is denoted as “40a-HIS”. This construct includes a GAS 40 sequence where the N-terminus leader sequence and the C-terminus containing the transmembrane sequence is removed. In this construct, the HIS tag sequence is added to the C-terminus (using for example, a cloning vector such as pET21b+ (Novagen) at the NdeI and NotI restriction sites). Polynucleotide and amino acid sequences for 40a-HIS are shown in SEQ ID NOS. 21 and 22.

In addition to the addition of purification tags, recombinant expression may also be facilitated by optimising coding sequences to those more abundant or accessible to the recombinant host. For example, the polynucleotide sequence AGA encodes an arginine amino acid residue. Arginine may also be encoded by the polynucleotide sequence CTG. This CTG codon is preferred by the translational enzymes in *E. coli*. In the 40a-HIS polynucleotide sequence SEQ ID NO 21, a C-terminus CTG coding for arginine has been replaced with CGT.

The following codons are generally underrepresented in *E.coli*: AGA, AGG and CGA. When these codons occur in a GAS polynucleotide sequence, they may be replaced with one of the other two optional codons encoding for the same amino acid residue.

A total of three ATG codons are optimised to CTG in the “40a-RR-HIS” construct, SEQ ID NOS 23 and 24. SEQ ID NO 23 is also shown below, with the optimised codons underlined. (other than the additional codon optimisation, 40a-RR-HIS is identical to 40a-HIS.)

SEQ ID N: 23

ATGAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGGCGAGTAATACTCACGACC
 ATAGTTTACCAAAACCAGAAACAATTCAAGAGGCAAAGGCAACTATTGATGCAGTTGAAAAAACTCTCAGTCAACAAAA
 AGCAGAACTGACAGAGCTTGCTACCGCTCTGACAAAACTACTGCTGAAATCAACCACCTTAAAAGAGCAGCAAGATAAT
 GAACAAAAGCTTTAACCTCTGCACAAGAAATTTACACTAATACTCTTGCAAGTAGTGAGGAGACGCATTAGCCCAAG
 GAGCCGAACATCAAAGAGAGTTAACAGCTACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGAC
 TGCATTGTCAGAACAAAAGCTAGCATTTTCAGCAGAAACTACTCGAGCTCAAGATTTAGTGGAACAAGTCAAAACGCTCT
 GAACAAAATATTGCTAAGCTCAATGCTATGATTAGCAATCCTGATGCTATCACTAAAGCAGCTCAAACGGCTAATGATA
 ATACAAAAGCATTAAGCTCAGAATTGGAGAAGGCTAAAGCTGACTTAGAAAATCAAAAAGCTAAAGTTAAAAGCAATT

5 GACTGAAGAGTTGGCAGCTCAGAAAAGCTGCTCTAGCAGAAAAAGAGGCAGAAGCTTAGTCGTCCTTAAATCCTCAGCTCCG
 TCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCGCAAGGCTATCCTCTTGAAGAACTTAAAAAATTAG
 AAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACAAAGAGCATGCAGATCAAATTATTGCCAAAGCTAG
 10 TCCAGGTAATCAATTAATCAATACCAAGATATTCAGCAGATCGTAATCGCTTTGTTGATCCCGATAATTTGACACCA
 GAAGTGCAAAATGAGCTAGCGCAGTTTGCAGCTCACATGATTAATAGTGTAcGtcGtCAATTAGGTCTACCACCAGTTA
 CTGTTACAGCAGGATCACAAGAAATTTGCAAGATTACTTAGTACCAGCTATAAGAAAACTCATGGTAATACAAGACCATC
 ATTTGTCTACGGACAGCCAGGGGTATCAGGGCATTATGGTGTGGGCCCTCATGATAAACTATTATTGAAGACTCTGCC
 GGAGCGTCAGGGCTCATTGCAAAATGATGATAACATGTACGAGAATATCGGTGCTTTTAAACGATGTGCACTACTGTGAATG
 GTATTAACCGTGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAAATACATACGGCCATGC
 15 TATTAACCTTTTTACGTGTAGATAAACATAACCCTAATGCGCCTGTTTACCTTGGATTTTCAACCAGCAATGTAGGATCT
 TTGAATGAACACTTTGTAATGTTCCAGAGTCTAACATTGCTAACCATCAACGCTTTAATAAGACCCCTATAAAAAGCCG
 TTGGAAGTACAAAAGATTATGCCCAAAGAGTAGGCCTGTATCTGATACTATTGCAGCGATCAAAGGAAAAGTAAGCTC
 ATTAGAAAATCGTTTGTGCGCTATTCATCAAGAAGCTGATATTATGGCAGCCCAAGCTAAAGTAAGTCAACTTCAAGGT
 AAATTAGCAAGCACACTTAAGCAGTCAGACAGCTTAAATCTCCAAGTGAGACAATTAATGATACTAAAGGTTCTTTGA
 20 GAACAGAATTAAGTACAGCTAAAAGCAAACAAGCACAACCTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATC
 GTTGAAAGCCGCACTGCACCAGACAGAAGCCTTAGCAGAGCAAGCCGACCCAGAGTGACAGCCTGGTGGCTAAAAAA
 GCTCATTGTGCAATATCTAAGGGACTTTAAATTGAATCCTAACCGCTTCAAGTGATACGTGAGCGCATTGATAATACATA
 AGCAAGATTTGGCTAAAACCTACCTCATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCTAAAACAAGCAG
 TCTAGAAGCTACTATTGCTACCACAGAACCAGTTGACTTTGCTTAAAACCTTAGCTAACGAAAAGGAATATCGCCAC
 25 TTAGACGAAGATATAGCTACTGTGCCGTGATTTGCAAGTAGCTCCACCTCTTACGGGCGTAAAACCGCTATCATATAGTA
 AGATAGATACTACTCCGCTTGTTCAGAAATGGTTAAAGAAAACGAAACAACCTATTAGAAGCTTCAGCAAGATTAGCTGC
 TGAAAATACAAGTCTTGTAGCAGAAGCGCTTGTGGCCAAACCTCTGAAATGGTAGCAAGTAATGCCATTGTGTCTAAA
 ATCACATCTTCGATTACTCAGCCCTCATCTAAGACATCTTATGGCTCAGGATCTTCTACAACGAGCAA.TCTCATTTCTG
 ATGTTGATGAAAGTACTCAAcGtGCGGCCGCACTCGAGCACCACCACCACCACCACC

30 Codon optimisation can also be used without a purification tag. Construct “40a-RR-Nat”, SEQ ID
 NOS: 25 and 26, provides such an example. This construct comprises GAS 40 without the N-terminus
 leader sequence and the C-terminus transmembrane sequence, with three codon optimisations (and does not
 include a HIS tag sequence).

35 Different cloning vectors can be used to optimise expression in different host cells or under
 different culture conditions. The above discussed constructs used pET21b+ (Novagen) vector which
 includes an IPTG inducible promoter. As an alternative, an *E.coli/B.subtilis* expression shuttle vector
 such as pSM214gNH may be used. This vector uses a constitutive promoter instead of an IPTG
 inducible promoter. An example of a GAS 40 construct using this vector is denoted as “HIS-40a-NH”,
 40 SEQ ID NOS 27 and 28. In this construct, both the N-terminus leader sequence and the C-terminus
 transmembrane sequence are removed, and a HIS tag is added to the N-terminus. Additional N-
 terminus amino acids are introduced with the cloning. In addition, two nucleotide changes which most
 likely occurred during PCR are indicated – neither of these changes results in amino acid changes.

45 As another alternative, the pSM214gCH shuttle vector may be used. An example of a GAS 40
 construct using this vector is denoted as “HIS-40a-CH”, SEQ ID NOS: 29 and 30. In this construct, the
 N-terminus leader sequence and the C-terminus transmembrane sequence are removed and the HIS tag
 is placed at the C-terminus. Two additional amino acids are also introduced at the amino terminus.
 Three nucleotide changes introduced with the cloning are shown in the DNA sequence, with a resulting
 amino acid change indicated in the protein sequence (from amino acid F to S).

Codon optimisation can also be used with these alternative cloning vectors. GAS 40 construct
 “HIS- 40a-RR-NH” comprises the “HIS-40a-NH” construct with three codon optimisations. HIS-40a-
 RR-NH is set forth in the sequence listing as SEQ ID NOS: 31 and 32.

Accordingly, the GAS antigens used in the invention may be produced recombinantly using expression constructs which facilitate their recombinant production. Preferred sequence modifications to facilitate expression may be selected from the group consisting of (1) the addition of a purification tag sequence and (2) codon optimisation.

5 As discussed above, Applicants have identified GAS 40 as being particularly suitable for use in immunogenic compositions, either alone or in combinations. The use of GAS 40 as a particularly effective GAS antigen is supported by its association with virulence, its surface localization, its effectiveness in bacterial opsonophagocytosis assays and in immunization challenge experiments. In addition, the potential horizontal acquisition of this virulence factor indicates that this antigen may be specific to GAS (relative to other Streptococcal bacteria). Further support for the antigenic properties of GAS 40 also includes the identification of coiled-coil regions within the GAS 40 two dimensional structure, and the low level homology of these regions with surface proteins of other Streptococcal bacteria, including some adhesion proteins.

15 Applicants' analysis of the location of GAS 40 within the *Streptococcal pyogenes* genome indicates that this virulence factor was likely acquired by GAS during evolution as a result of a horizontal gene transfer. Figure 5A depicts GAS 40 within the GAS genome. It is preceded on the 5' end by a sequence designated "purine operon repressor" or "purR". It is followed on the 3' end by two sequences encoding ribosomal proteins designated "ribosomal protein S12", or "rpsL" and "ribosomal protein S7" or "rpsG". (Amino acid and polynucleotide sequences for these flanking genes are publicly available on GenBank. (PurR sequences can be found for example under Genbank reference GI:15674250. RpsL sequences can be found for example under Genbank reference GI:15674250. RpsG sequences can be found for example under Genbank reference GI:15674250. Notably, there are two putative promoter sequences designated at the beginning of the rpsL sequence. Figure 5B depicts a GAS mutant where a large portion of GAS 40 is deleted. The only portion of the GAS 40 sequence remaining corresponds to polynucleotides 1 – 97 of SEQ ID NO: 2. The deletion included one of the rpsL promoters, leaving the second, P*, intact. (The horizontal arrows underlining the schematic indicate the deleted region.)

25 Figure 5C provides additional detail on the wildtype GAS sequence. Here, direct repeat sequences, designated "DR", are shown flanking the 5' and 3' ends of GAS 40. (The corresponding sequences in the GAS 40 deletion mutant are identified in Figure 5D). These direct repeat sequences are approximately 8 basepairs. One example of such a basepair direct repeat comprises SEQ ID NO: 136. Such sequence motifs within a bacterial genome frequently indicate a horizontal gene transfer. *In vivo* infection experiments show that the GAS 40 deletion mutant is several logs less virulent than the wild type strain. (Details of this experiment are provided in Example 2).

35 The combination of the presence of the flanking direct repeat sequences and the virulence associated with GAS 40 strongly suggests that the GAS 40 sequence was horizontally acquired by *Streptococcus pyogenes* during evolution. Notably, while related purR and rpsL are present in related

Streptococcal bacteria *Streptococcus agalactiae* and *Streptococcus mutants*, neither of these bacteria are known to have a GAS 40 homologue. (Figure 5E schematically depicts the location of *purR*, *rpsL*, and *rpsG* homologues within *S. agalactiae* (Group B Streptococcus) and shows the percent homology of the GBS homologues with the GAS counterparts. Notably, GBS genomes generally only possess one of the direct repeat sequences – and do not contain a pair of the direct repeat sequences flanking the GAS 40 sequence.)

The surface location of GAS 40 is illustrated by the FACS diagram presented in Figure 6. (Discussion of protocols relating to FACS analysis is presented in Example 1). Figure 6 includes FACS diagrams for both the wild type GAS (designated DSM 2071, an M23 type of GAS) and the deletion mutant (designated DSM 2071 Δ 40). The absorbance shift for the wild type strain indicates that GAS 40 is recognized on the surface of the bacteria by anti-GAS 40 antibodies (and that it is not recognized on the surface of the deletion mutant).

The surface exposure of GAS 40 is further demonstrated by a bacterial opsonophagocytosis assay illustrated in Figure 7 and in Example 3. In this assay, GAS strains are incubated with preimmune and immune sera, polymorphonucleates and complement. (The immune sera is generated by mouse immunization with the indicated GAS protein.) Phagocytosis or growth of the bacteria are measured logarithmically. Positive histogram bars represent phagocytosis (or bacterial death). Negative histogram bars represent bacterial growth. As shown in Figure 7, immune sera generated by each of the GAS40 expressed proteins resulted in a reduction of bacteria (positive histogram bars).

Immunization challenge studies with GAS 40 are discussed in detail in Example 4. As shown in this example, GAS 40, as produced using various constructs, provides substantial protection in adult mice. Notably, most GAS40 constructs provide almost as much protection as GAS M protein. (GAS M protein is used for comparison as it is known to be highly immunogenic. However, M protein is generally not regarded as a suitable GAS vaccine candidate as it varies widely among GAS strains and has epitopes with potential cross-reactivity with human tissues.) In addition, an N-terminus fragment of GAS 40 also provided significant protection in this model. The N-terminus fragment comprises about 292 amino acids from the N-terminus of GAS 40 overlaps with the first coiled-coil region. “40N-HIS” (SEQ ID NOS. 33 and 34) is an example of this GAS 40 fragment which comprises the coiled-coil region of GAS 40 and a C-terminus HIS tag.

30 (2) GAS 117

GAS 117 corresponds to M1 GenBank accession numbers GI:13621679 and GI:15674571, to M3 GenBank accession number GI:21909852, to M18 GenBank accession number GI: 19745578, and is also referred to as ‘Spy0448’ (M1), ‘SpyM3_0316’ (M3), and ‘SpyM18_0491’ (M18). Examples of amino acid and polynucleotide sequences of GAS 117 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 35 and 36.

Preferred GAS 117 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99%, 99.5% or more) to SEQ ID NO: 35; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 35, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These GAS 117 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 35. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 1. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 35. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 35 (shown below) is removed. (SEQ ID NO: 37 comprises the removed N-terminal amino acid sequence. SEQ ID NO: 38 comprises a fragment of GAS 117 without the N-terminal amino acid sequence). Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 35

MTLKKHYLLSLALVTVGAAFNNTSQSVSAQVYSNEGYHQHLTDEKSHLQYSKDNAQLQLRNILDGYQNDLGRHYSSYYY
YNLRTVMGLSSEQDIEKHYEELKNKLHDMYNYH

(3) GAS 130

GAS 130 corresponds to M1 GenBank accession numbers GI:13621794 and GI:15674677, to M3 GenBank accession number GI: 21909954, to M18 GenBank accession number GI: 19745704, and is also referred to as 'Spy0591' (M1), 'SpyM3_0418' (M3), and 'SpyM18_0660' (M18). GAS 130 has potentially been identified as a putative protease. Examples of amino acid and polynucleotide sequences of GAS 130 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 39 and 40.

Preferred GAS 130 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 39; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 39, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 130 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 39. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 39. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 39. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(4) GAS 277

GAS 277 corresponds to M1 GenBank accession numbers GI:13622962 and GI:15675742, to M3 GenBank accession number GI: 21911206, to M18 GenBank accession number GI: 19746852, and is also referred to as 'Spy1939' (M1), 'SpyM3_1670' (M3), and 'SpyM18_2006' (M18). Amino acid and polynucleotide sequences of GAS 277 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 41 and 42.

Preferred GAS 277 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 41; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 41, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 277 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 41. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 41. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 41. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 41 (shown below) is removed. (SEQ ID NO: 43 comprises the underlined N-terminal amino acid. SEQ ID NO: 44 comprises a fragment of GAS 277 with the N-terminal amino acid sequence removed). Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

15 **SEQ ID NO: 41**

MTTMOQKTISLLSLALLIGLLGTSGKAISVYAQDQHTDNVIAESTISQVSVEASMRGTEPYIDATVTTDQPVRRQPTQATIT
LKDASDNTTINSWVYTMAAQRRFTAWFDLTGQKSGDYHVTVTVHTQEKAVTGQSGTVHFDQNKARKTPTNMQQKDTSKAM
TNSVDVDTKAQTNQSANQEIDSTSNPFRSATNHRSTSLKRSTKNEKLTPTASNSQKNGSNKTKMLVDKEEVKPTSKRGFP
WVLLGLVVSLAAGLF~~I~~AIQKVSRRK

20

(5) **GAS 236**

GAS 236 corresponds to M1 GenBank accession numbers GI:13622264 and GI:15675106, M3 GenBank accession number GI: 21910321, and to M18 GenBank accession number GI: 19746075, and is also referred to as 'Spy1126' (M1), 'SpyM3_0785' (M3), and 'SpyM18_1087' (M18). Amino acid and polynucleotide sequences of GAS 236 from an M1 strain are set forth in the sequence listing as SEQ ID NOS: 45 and 46.

Preferred GAS 236 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 45; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 45, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 236 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 45. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 45. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 45. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 45 (shown below) is removed. (SEQ ID NO: 47 comprises the N-terminus amino acid sequence. SEQ ID NO: 48 comprises a fragment of GAS 236 with the N-terminus sequence removed). Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

40 **SEQ ID NO: 45**

MTQMNYTGKVKRVAIIANGKYQSKRVASKLFSVFKDDPDFYLSKKNPDIVISIGGDGMLLSAFHMYEKELDKVRFVGIHT
GHLGFYTDYRDFEVDKLI~~DN~~LRKDKGEQISYPILKVAITLDDGRVVKARALNEATVKRIEKT~~M~~VADVII~~NH~~VK~~F~~ESFRGD

GISVSTPTGSTAYNKSLGGAVLHPTIEALQLTEISSLNRRVFRTLGSSIIIPKKDKIELVPKRLGIYTTISIDNKTYQLKN
 VTKVEYFIDDEKIHVSSPSTSFWERVKDAFIGEIDS

(6) GAS 389

5 GAS 389 corresponds to M1 GenBank accession numbers GI:13622996 and GI:15675772, to M3
 GenBank accession number GI: 21911237, to M18 GenBank accession number GI: 19746884, and is also
 referred to as 'Spy1981' (M1), 'SpyM3_1701' (M3), 'SpyM18_2045' (M18) and 'relA'. GAS 389 has also
 been identified as a (p)ppGpp synthetase. Amino acid and polynucleotide sequences of GAS 389 from an
 M1 strain are set forth in the sequence listing as SEQ ID NOS: 49 and 50.

10 Preferred GAS 389 proteins for use with the invention comprise an amino acid sequence: (a) having
 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
 98%, 99%, 99.5% or more) to SEQ ID NO: 49; and/or (b) which is a fragment of at least *n* consecutive
 amino acids of SEQ ID NO: 49, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60,
 70, 80, 90, 100, 150, 200, 250 or more). These GAS 389 proteins include variants (*e.g.* allelic variants,
 15 homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 49. Preferred fragments of (b) comprise an
 epitope from SEQ ID NO: 49. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6,
 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8,
 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 49. Other fragments omit one or more
 domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane
 20 domain, or of an extracellular domain).

(7) GAS 504

GAS 504 corresponds to M1 GenBank accession numbers GI:13622806 and GI:15675600, to M3
 GenBank accession number GI: 21911061, to M18 GenBank accession number GI: 19746708, and is also
 referred to as 'Spy1751' (M1), 'SpyM3_1525', 'SpyM18_1823' (M18) and 'fabK'. GAS 504 has also been
 25 identified as a putative trans-2-enoyl-ACP reductase II. Amino acid and polynucleotide sequences of GAS
 504 of an M1 strain are set forth below and in the sequence listing as SEQ ID NOS: 51 and 52.

Preferred GAS 504 proteins for use with the invention comprise an amino acid sequence: (a) having
 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
 98%, 99%, 99.5% or more) to SEQ ID NO: 51; and/or (b) which is a fragment of at least *n* consecutive
 30 amino acids of SEQ ID NO: 51, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60,
 70, 80, 90, 100, 150 or more). These GAS 504 proteins include variants (*e.g.* allelic variants, homologs,
 orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 51. Preferred fragments of (b) comprise an epitope from
 SEQ ID NO: 51. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15,
 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20,
 35 25 or more) from the N-terminus of SEQ ID NO: 51. Other fragments omit one or more domains of the
 protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an
 extracellular domain).

(8) GAS 509

GAS 509 corresponds to M1 GenBank accession numbers GI:13622692 and GI:15675496, to M3
 40 GenBank accession number GI: 21910899, to M18 GenBank accession number GI: 19746544, and is also
 referred to as 'Spy1618' (M1), 'SpyM3_1363' (M3), 'SpyM18_1627' (M18) and 'cysM'. GAS 509 has

also been identified as a putative O-acetylserine lyase. Amino acid and polynucleotide sequences of GAS 509 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 53 and 54.

Preferred GAS 509 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 53; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 53, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 509 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 53. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 53. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 53. For example, in one embodiment, the underlined amino acid sequence at the C-terminus of SEQ ID NO: 53 (shown below) is removed. (SEQ ID NO: 55 comprises the C-terminus amino acid sequence. SEQ ID NO: 56 comprises a fragment of GAS 509 with the C-terminus sequence removed). Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 53

MTKIYKTITELVGQTPPIIKLNRLIPNEAADVYVKLEAFNPGSSVKDRIALSMTEAAEAEGLISPGDVII
PTSGNTGIGLAWVGAAGYRVIIIVMPETMSLERRQIIQAYGAELVLTPGAEGMKGAIKAETLAIELGAW
MPMQFNPNANPSIHEKTTAQETLEAFKEISLDAFVSGVGTGGTILSGVSHVLKKNPETVIYAVEAEESAV
LSGQEPGPHKIQQISAGFIPTLDTKAYDQIIRVKSKDALETARLTGAKEGFLVGISSGAALYAAIEVAK
QLGKGGKHVLTILPDNGERYLSTELYDVPVIKTK

(9) GAS 366

GAS 366 corresponds to M1 GenBank accession numbers GI:13622612, GI:15675424 and GI:30315979, to M3 GenBank accession number GI: 21910712, to M18 GenBank accession number GI: 19746474, and is also referred to as 'Spy1525' (M1), 'SpyM3_1176' (M3), 'SpyM18_1542' (M18) and 'murD'. GAS 366 has also been identified as a UDP-N-acetylmuramoylalanine-D-glutamate ligase or a D-glutamic acid adding enzyme. Amino acid and polynucleotide sequences of GAS 366 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 57 and 58.

Preferred GAS 366 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 57; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 57, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 366 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 57. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 57. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 57. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 57 (shown below) is removed. (SEQ ID NO: 59 comprises the N-terminus leader sequence. SEQ ID NO: 60 comprises a fragment of GAS 366 where the N-terminus sequence is removed). Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 57

MKVISNFQNKILILGLAKSGEAAAKLLTKLGALVTVNDSKPFQNPAAQALLEEGIKVICGSHPVLELLDENFEYMVKNP
GIPYDNPVMKRALAKEIPILTEVELAYFVSEAPIIGITGSNGKTTTTTMIADVLNAGGQSALLSGNIGYPASKVVQKATA

GDTLVMELSSFQLVGVNAFRPHIAVITNLMPTHLDYHGFSFEDYVAAKMMIQAQMTESDYLLILNANQEI SATLAKTTKATV
 IPFSTQKVVVDGAYLKDGI LYFKEQAI IAAATDLGVPGSHNIENALATI AVAKLSGIADDI IAQCLSHFGGVKHLRQRVGQI
 KDITFYNDKSKSTNILATQKALSGFDNSRLIL IAGGLDRGNEFDDLPDLLGLKQMI ILGESAERMKRAANKAEVSYLEAR
 NVAEATELAFKLAQTGDTILLSPANASWDMYPNFEVRGDEFLATFDCLRGDA

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(10) GAS 159

AS 159 corresponds to M1 GenBank accession numbers GI:13622244 and GI:15675088, to M3 GenBank accession number GI: 21910303, to M18 GenBank accession number GI: 19746056, and is also referred to as 'Spy1105' (M1), 'SpyM3_0767' (M3), 'SpyM18_1067' (M18) and 'potD'. GAS 159 has also been identified as a putative spermidine/putrescine ABC transporter (a periplasmic transport protein). Amino acid and polynucleotide sequences of GAS 159 of an M1 strain are set forth below and in the sequence listing as SEQ ID NOS: 61 and 62.

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SEQ ID NO: 61

MRKLYSFLAGVLGVIVILTSLSFILQKKSQSDKLVIIYNWGDYIDPALLKKFKTKETGIEVQYETFD SNEAMYTKIKQ
 GGTTYDIAVPSDYTIDKMIKENLLNKLDKSKLVGMDNIGKEFLGKSFDPQNDYSLPYFWGTVGIVYNDQLVDKAPMHWED
 LWRPEYKNSIMLIDGAREMLGVGLTTFGYSVNSKNLEQLQAAERKLQQLTPNVKAI VADEMKGMIQGDAAIGITFSGEA
 SEMLDSNEHLHYIVPSEGSNLWFDNLVLPKTMKHEKEAYAF LNF INRPENAAQNAAYIGYATPNKKAKALLPDEIKNDPA
 FYPTDDIIKKLEVDNLGSRWLGIIYNDLYLQFKMYRK

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SEQ ID NO: 62

ATGCGTAAACTTTATTCCTTTCTAGCAGGAGTTTTGGGTGTTATTTGTTATTTTAAACAAGTCTTTCTTTTCATCTTGCAGAA
 AAAATCGGGTCTGGTAGTCAATCGGATAAAATTAGTTATTTATAACTGGGGAGATTACATTGATCCAGCTTTGCTCAAAA
 AATTCACCAAAGAAACGGGCATTGAAGTGCAGTATGAAACTTTCGATCCAATGAAGCCATGTACACTAAAATCAAGCAG
 GGCGGAACCACTTACGACATTGCTGTTCCCTAGTGATTACACCATTGATAAAAATGATCAAAGAAAACCTACTCAATAAGCT
 TGATAAGTCAAAAATTAGTTGGCATGGATAATATCGGGAAAAGAAATTTT TAGGGAAAAGCTTTGACCCACAAAACGACTATT
 CTTTGCCTTATTTCTGGGGAACCGTTGGGATTGTTTATAATGATCAATTAGTTGATAAGGCGCCTATGCATGGGAAGAT
 CTGTGGCGTCCAGAATAAAAAATAGTATTATGCTGATTGATGGAGCGGTGAAATGCTAGGGGTTGGTTTAAACAACCTTT
 TGGTTATAGTGTGAATCTAAAAATCTAGAGCAGTTGCAGGCAGCCGAGAGAAAAC TGCAGCAGTTGACGCCGAATGTTA
 AAGCCATTGTAGCAGATGAGATGAAAGGCTACATGATTCAAGGTGACGCTGCTATTGGAATTACCTTTTCTGGTGAAGCC
 AGTGAGATGTTAGATAGTAACGAACACCTTCACTACATCGTGCCCTCAGAAGGGTCTAACCTTTGGTTTGATAATTTGGT
 ACTACCAAAAACCATGAAACACGAAAAAGAAGCTTATGCTTTTTTGAACCTTATCAATCGTCTGAAAATGCTGCGCAA
 ATGCTGCATATATTGGTTATGCGACACCAATAAAAAAGCCAAGGCTTACTTCCAGATGAGATAAAAAATGATCC TGCT
 TTTTATCCAACAGATGACATTATCAAAAAATTGGAAGTTTATGACAATTTAGGGTCAAGATGGTTGGGGATTTATAATGA
 TTTTATACCTCCAATTTAAAAATGTATCGCAAATAA

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Preferred GAS 159 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 61; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 61, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 159 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 61. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 61. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 61. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 61 (shown below) is removed. (SEQ ID NO: 63 comprises the N-terminus leader amino acid sequence. SEQ ID NO: 64 comprises a fragment of GAS 159 where the N-terminus leader amino acid sequence is removed). In another example, the underlined amino acid sequence at the C-terminus of SEQ ID NO: 61 is removed. (SEQ ID NO: 65 comprises the C-terminus hydrophobic region. SEQ ID NO: 66 comprises a fragment of GAS 159 where the C-terminus hydrophobic region is removed. SEQ ID NO: 67 comprises a fragment of GAS 159 where both the N-terminus leader sequence and C-terminus hydrophobic region are removed.) Other fragments omit one or more domains of

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the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 61

MRKLYSFLAGVLGVIVILTSLSLFLLQKKSGSGSQSDKLVIIYNWGDYIDPALLKKFTKETGIEVQYETFDSSNEAMYTKIKQ
 5 GGTTYDIAVPSDYTIKMIKENLLNKLDKSKLVGMDNIGKEFLGKSFDPQNDYSLPYFWGTGIVYNDQLVDKAPMHWED
 LWRPEYKNSIMLIDGAREMLGVGLTTFGYSVNSKNLEQLQAAERKLQQLTPNVKAIVADEMKGYMIQGDAAIIGITFSGEA
 SEMLDSNEHLHYIVPSEGSNLWFDNLVLPKTMKHEKEAYAFNLFINRPENAAQNAAAYIGYATPNKKAKALLPDEIKNDPZA
 FYPTDDI IKKLEVDNLGSRWLG IYNDLYLQFKMYRK

10 **(11) GAS 217**

GAS 217 corresponds to M1 GenBank accession numbers GI:13622089 and GI:15674945, to M3 GenBank accession number GI: 21910174, to M18 GenBank accession number GI: 19745987, and is also referred to as 'Spy0925' (M1), 'SpyM3_0638' (M3), and 'SpyM18_0982' (M18). GAS 217 has also been identified as a putative oxidoreductase. Amino acid and polynucleotide sequences of GAS 217 of an M1
 15 strain are set forth in the sequence listing as SEQ ID NOS: 68 and 69.

Preferred GAS 217 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 68; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 68, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60,
 20 70, 80, 90, 100, or more). These GAS 217 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 68. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 68. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 68. Other fragments omit one or more domains of the
 25 protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(12) GAS 309

GAS 309 corresponds to M1 GenBank accession numbers GI:13621426 and GI:15674341, to M3 GenBank accession number GI: 21909633, to M18 GenBank accession number GI: 19745363, and is also referred to as 'Spy0124' (M1), 'SpyM3_0097' (M3), 'SpyM18_0205' (M18), 'nra' and 'rofA'. GAS 309 has also been identified as a regulatory protein and a negative transcriptional regulator. Amino acid and polynucleotide sequences of GAS 309 of an M1 strain are set forth in the sequence listing as SEQ ID NOS:
 30 70 and 71.

Preferred GAS 309 proteins for use with the invention comprise an amino acid sequence: (a) having 35 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 70; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 70, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 309 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 70. Preferred fragments of (b) comprise an epitope from
 40 SEQ ID NO: 70. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 70. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(13) GAS 372

GAS 372 corresponds to M1 GenBank accession numbers GI:13622698 and GI:15675501, to M3 GenBank accession number GI: 21910905, to M18 GenBank accession number GI: 19746500 and is also referred to as 'Spy1625' (M1), 'SpyM3_1369' (M3), and 'SpyM18_1634' (M18). GAS 372 has also been identified as a putative protein kinase or a putative eukaryotic-type serine/threonine kinase. Amino acid and polynucleotide sequences of GAS 372 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 72 and 73.

Preferred GAS 372 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 72; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 72, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These GAS 372 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 72. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 72. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 72. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(14) GAS 039

GAS 039 corresponds to M1 GenBank accession numbers GI:13621542 and GI:15674446, to M3 GenBank accession number GI: 21909730, to M18 GenBank accession number GI: 19745398 and is also referred to as 'Spy0266' (M1), 'SpyM3_0194' (M3), and 'SpyM18_0250' (M18). Amino acid and polynucleotide sequences of GAS 039 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 74 and 75.

Preferred GAS 039 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 74; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 74, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 039 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 74. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 74. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 74. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(15) GAS 042

GAS 042 corresponds to M1 GenBank accession numbers GI:13621559 and GI:15674461, to M3 GenBank accession number GI: 21909745, to M18 GenBank accession number GI: 19745415, and is also referred to as 'Spy0287' (M1), 'SpyM3_0209' (M3), and 'SpyM18_0275' (M18). Amino acid and polynucleotide sequences of GAS 042 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 76 and 77.

Preferred GAS 042 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 76; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 76, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 042 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 76. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 76. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 76. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(16) GAS 058

GAS 058 corresponds to M1 GenBank accession numbers GI:13621663 and GI:15674556, to M3 GenBank accession number GI: 21909841, to M18 GenBank accession number GI: 19745567 and is also referred to as 'Spy0430' (M1), 'SpyM3_0305' (M3), and 'SpyM18_0477' (M18). Amino acid and polynucleotide sequences of GAS 058 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 78 and 79.

Preferred GAS 058 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 78; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 78, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 058 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 78. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 78. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 78. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 78 (shown below) is removed. (SEQ ID NO: 80 comprises the N-terminal leader sequence. SEQ ID NO: 81 comprises a fragment of GAS 58 where the N-terminal leader sequence is removed.) Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 78

MKWSGFMKTKSKRFLNLATLCLALLGTTLLMAHPVQAEVISKRDYMTRFGLGDLEDDSANYPSNLEARYKGYLEGEKGL
 KGDDI PERPKIQVPEDVQPSDHGDYRDGYEEGFGEQHKRDPLETEAEDDSQGGRQEGRQGHQEGADSSDLNVEESDGLS
 VIDEVVGVIYQAFSTIWTYLSGLF

(17) GAS 290

GAS 290 corresponds to M1 GenBank accession numbers GI:13622978 and GI:15675757, to M3 GenBank accession number GI: 21911221, to M18 GenBank accession number GI: 19746869 and is also referred to as 'Spy1959' (M1), 'SpyM3_1685' (M3), and 'SpyM18_2026' (M18). Amino acid and polynucleotide sequences of GAS 290 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 82 and 83.

Preferred GAS 290 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 82; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 82, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These GAS 290 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 82. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 82. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 82. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(18) GAS 511

GAS 511 corresponds to M1 GenBank accession numbers GI:13622798 and GI:15675592, to M3 GenBank accession number GI: 21911053, to M18 GenBank accession number GI: 19746700 and is also referred to as 'Spy1743' (M1), 'SpyM3_1517' (M3), 'SpyM18_1815' (M18) and 'acca'. Amino acid and polynucleotide sequences of GAS 511 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 84 and 85.

Preferred GAS 511 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 84; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 84, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These GAS 511 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 84. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 84. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 84. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(19) GAS 533

GAS 533 corresponds to M1 GenBank accession numbers GI:13622912 and GI:15675696, to M3 GenBank accession number GI: 21911157, to M18 GenBank accession number GI: 19746804 and is also referred to as 'Spy1877' (M1), 'SpyM3_1621' (M3), 'SpyM18_1942' (M18) and 'glnA'. GAS 533 has also been identified as a putative glutamine synthetase. Amino acid and polynucleotide sequences of GAS 533 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 86 and 87.

Preferred GAS 533 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 86; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 86, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 533 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 86. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 86. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20,

25 or more) from the N-terminus of SEQ ID NO: 86. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(20) GAS 527

5 GAS 527 corresponds to M1 GenBank accession numbers GI:13622332, GI:15675169, and GI:24211764, to M3 GenBank accession number GI: 21910381, to M18 GenBank accession number GI: 19746136, and is also referred to as 'Spy1204' (M1), 'SpyM3_0845' (M3), 'SpyM18_1155' (M18) and 'guaA'. GAS 527 has also been identified as a putative GMP synthetase (glutamate hydrolyzing) (glutamate amidotransferase). Amino acid and polynucleotide sequences of GAS 527 of an M1 strain are set forth in
10 the sequence listing as SEQ ID NOS: 88 and 89.

Preferred GAS 527 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 88; and/or (b) which is a fragment of at least *n* consecutive
15 amino acids of SEQ ID NO: 88, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 527 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 88. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 88. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15,
20 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 88. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(21) GAS 294

GAS 294 corresponds to M1 GenBank accession numbers GI:13622306, GI:15675145, and GI:26006773, to M3 GenBank accession number GI: 21910357, to M18 GenBank accession number GI:
25 19746111 and is also referred to as 'Spy1173' (M1), 'SpyM3_0821' (M3), 'SpyM18_1125' (M18) and 'gid'. GAS 294 has also been identified as a putative glucose-inhibited division protein. Amino acid and polynucleotide sequences of GAS 294 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 90 and 91.

Preferred GAS 294 proteins for use with the invention comprise an amino acid sequence: (a) having
30 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 90; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 90, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 294 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 90. Preferred fragments of (b) comprise an epitope from
35 SEQ ID NO: 90. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 90. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(22) GAS 253

GAS 253 corresponds to M1 GenBank accession numbers GI:13622611, GI:15675423, and GI:21362716, to M3 GenBank accession number GI: 21910711, to M18 GenBank accession number GI: 19746473 and is also referred to as 'Spy1524' (M1), 'SpyM3_1175' (M3), 'SpyM18_1541' (M18) and 'murG'. GAS 253 has also been identified as a putative undecaprenyl-PP-MurNAc-pentapeptide-UDPGlcNAc GlcNAc transferase. Amino acid and polynucleotide sequences of GAS 253 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 92 and 93.

Preferred GAS 253 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 92; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 92, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 253 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 92. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 92. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 92. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(23) GAS 529

GAS 529 corresponds to M1 GenBank accession numbers GI:13622403, GI:15675233, and GI:21759132, to M3 GenBank accession number GI: 21910446, to M18 GenBank accession number GI: 19746203 and is also referred to as 'Spy1280' (M1), 'SpyM3_0910' (M3), 'SpyM18_1228' (M18) and 'glmS'. GAS 529 has also been identified as a putative L-glutamine-D-fructose-6-phosphate aminotransferase (Glucosamine-6-phosphate synthase). Amino acid and polynucleotide sequences of GAS 529 of an M1 strain are set forth below and in the sequence listing as SEQ ID NOS: 94 and 95.

Preferred GAS 529 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 94; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 94, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 529 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 94. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 94. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 94. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(24) GAS 045

GAS 045 corresponds to M3 GenBank accession number GI: 21909751, M18 GenBank accession number GI: 19745421 and is referred to as 'SpyM3_0215' (M3), 'SpyM18_oppA' (M18) and 'oppA'. GAS 045 has been identified as an oligopeptide permease. Amino acid and polynucleotide sequences of GAS 045 from an M1 strain are set forth in the sequence listing as SEQ ID NOS: 96 and 97.

Preferred GAS 045 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 96; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 96, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 045 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 96. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 96. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 96. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 96 (shown below) is removed. (SEQ ID NO: 98 comprises the underlined N-terminal leader sequence. SEQ ID NO: 99 comprises a fragment of GAS 45 where the N-terminal leader sequence is removed). Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 96

VTFMKKSKWLA~~AAVS~~VAILS~~SVS~~SALAAACGNKNASGGSEATKTYKYVVFVNDPKSLDYILTN~~GGG~~TTD~~VIT~~QMV~~DGL~~LEND~~EY~~GNL~~VPS~~LAKDWK~~VSK~~DGLTYTYTL~~RD~~GVSWYTADG~~EYA~~PVTAEDFVTGLKHAVDDKSDALYVVEDS~~IKNL~~KAYQNGEVDFKEVGVKALDDKTVQYTLNKPESYWN~~SK~~TTYSVLF~~FP~~VNAKFLKSKGKDFGTTDPSSILVNGAYFLSAFTSKSSMEFHK~~NYEN~~YWD~~AK~~NVGI~~ES~~VKLTYS~~SD~~GSDPGSFYK~~NF~~DKGEFSVARLYPNDPTYKS~~AK~~KNYADNITYGMLTGD~~IR~~H~~L~~TWNLN~~R~~TSFKN~~TK~~KDPAQQDAGK~~KAL~~NNKDFRQAIQFAFDRAS~~FQA~~QTAGQDAKTKALRNMLVPPTFVTTIGESDFGSEVEKEMAKLGDEWK~~DVN~~LADAQDGFYNPEKAKAEFAKAKEALTAEGVTF~~FP~~VQLDY~~PVD~~QANAATVQEAQSFKQ~~SVE~~ASLGKENVIVNVLETETS~~THE~~AQGFYAETPEQQDYDI~~ISS~~W~~W~~GPDYQDPRTYLDIMS~~PV~~GGGSVIQKLG~~IK~~AGQNKDVVAAAGLD~~TY~~QTL~~L~~DEAAAIT~~DD~~N~~D~~ARYKAYAKAQAYLTDNAVDI~~PV~~VALGGT~~PR~~VTKAVPFSSGGFSWAGSKGPLAYKGMKLQDKPVT~~VK~~QY~~EK~~AK~~EK~~W~~WK~~AKAKSNAKYAEKLADHVEK

(25) GAS 095

GAS 095 corresponds to M1 GenBank accession numbers GI:13622787 and GI:15675582, to M3 GenBank accession number GI: 21911042, to M18 GenBank accession number GI: 19746634 and is also referred to as 'Spy1733' (M1), 'SpyM3_1506' (M3), 'SpyM18_1741' (M18). GAS 095 has also been identified as a putative transcription regulator. Amino acid and polynucleotide sequences of GAS 095 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 100 and 101.

Preferred GAS 095 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 100; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 100, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 095 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 100. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 100. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15,

20, 25 or more) from the N-terminus of SEQ ID NO: 100. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 100 (shown below) is removed. (SEQ ID NO: 102 comprises the amino acid sequence of the underlined N-terminal leader sequence. SEQ ID NO: 103 comprises a fragment of GAS 95 where the N-terminal leader sequence is removed.) Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 100

MKIGKKIVLMFTAIVLTTVLALGVYLTSAYTFSTGELSKTFKDFSTSSNKSDAIKQTRAFSILLMGVDTGSSERASKWEG
 NSDSMILVTVNPKTKKTTMTSLERDTLTTLSGPKNNEMNGVEAKLNAAAYAAGGAQMAIMTVQDLLNITIDNYVQINMQGL
 IDLVNAVGGITVTNEFDFFPISIAENEPEYQATVAPGTHKINGEQALVYARMRYDDPEGDYGRQKRQREVIQKVLKKILAL
 DSISSYRKILSAVSSNMQTNIEISSRTIPSLGTYRDLRTIKTYQLKGEDATLSDGGSYQIVTSNHLLEIQNRIRTELGL
 HKVNQLKTNATVYENLYGSKTSQTVNNNYDSSGQAPSYSYSDSHSSYANYSSGVDTGQSASTDQDSTASSHRPATPSSSSDA
 LAADESSSSGSGSLVPPANINPQT

(26) GAS 193

GAS 193 corresponds to M1 GenBank accession numbers GI:13623029 and GI:15675802, to M3 GenBank accession number GI: 21911267, to M18 GenBank accession number GI: 19746914 and is also referred to as 'Spy2025' (M1), 'SpyM3_1731' (M3), 'SpyM18_2082' (M18) and 'isp'. GAS 193 has also been identified as an immunogenic secreted protein precursor. Amino acid and polynucleotide sequences of GAS 193 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 104 and 105.

Preferred GAS 193 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 104; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 104, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 193 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 104. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 104. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 104. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(27) GAS 137

GAS 137 corresponds to M1 GenBank accession numbers GI:13621842, GI:15674720 and GI:30173478, to M3 GenBank accession number GI:21909998, to M18 GenBank accession number GI: 19745749 and is also referred to as 'Spy0652' (M1), 'SpyM3_0462', and 'SpyM18_0713' (M18). Amino acid and polynucleotide sequences of GAS 137 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 106 and 107.

Preferred GAS 137 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 106; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 106, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 137 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 106. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 106. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 106. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

5 (28) **GAS 084**

GAS 084 corresponds to M1 GenBank accession numbers GI:13622398 and GI:15675229, to M3 GenBank accession number GI: 21910442, to M18 GenBank accession number GI: 19746199 and is also referred to as 'Spy1274' (M1), 'SpyM3_0906' and 'SpyM18_1223' (M18). GAS 084 has also been identified as a putative amino acid ABC transporter/periplasmic amino acid binding protein. Amino acid and polynucleotide sequences of GAS 084 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 108 and 109.

Preferred GAS 084 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 108; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 108, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 084 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 108. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 108. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 108. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 108 (shown below) is removed. (SEQ ID NO: 110 comprises an amino acid sequence comprising the underlined N-terminal leader sequence of GAS 84. SEQ ID NO: 111 comprises a fragment of GAS 84 where the N-terminal leader sequence is removed). Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 108

MI I K K R T V A I L A I A S S F F L V A C Q A T K S L K S G D A W G V Y Q K Q K S I T V G F D N T F V P M G Y K D E S G R C K G F D I D L A K E V F H Q Y G L
K V N F Q A I N W D M K E A E L N N G K I D V I W N G Y S I T K E R Q D K V A F T D S Y M R N E Q I I V V K K R S D I K T I S D M K H K V L G A Q S A S S G Y D
S L L R T P K L L K D F I K N K D A N Q Y E T F T Q A F I D L K S D R I D G I L I D K V Y A N Y Y L A K E G Q L E N Y R M I P T T F E N E A F S V G L R K E D K
T L Q A K I N R A F R V L Y Q N G K F Q A I S E K W F G D D V A T A N I K S

(29) **GAS 384**

GAS 384 corresponds to M1 GenBank accession numbers GI:13622908 and GI:15675693, to M3 GenBank accession number GI: 21911154, to M18 GenBank accession number GI: 19746801 and is also referred to as 'Spy1874' (M1), 'SpyM3_1618' (M3), and 'SpyM18_1939' (M18). GAS 384 has also been identified as a putative glycoprotein endopeptidase. Amino acid and polynucleotide sequences of GAS 384 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 112 and 113.

Preferred GAS 384 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 112; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 112, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 384 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 112. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 112. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 112. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

5 **(30) GAS 202**

GAS 202 corresponds to M1 GenBank accession numbers GI:13622431 and GI:15675258, to M3 GenBank accession number GI: 21910527, to M18 GenBank accession number GI: 19746290 and is also referred to as 'Spy1309' (M1), 'SpyM3_0991' (M3), 'SpyM18_1321' (M18) and 'dltD'. GAS 202 has also been identified as a putative extramembranal protein. Amino acid and polynucleotide sequences of GAS 202 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 114 and 115.

10 Preferred GAS 202 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 114; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 114, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 202 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 114. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 114. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 114. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

20 **(31) GAS 057**

GAS 057 corresponds to M1 GenBank accession numbers GI:13621655 and GI:15674549, to M3 GenBank accession number GI: 21909834, to M18 GenBank accession number GI: 19745560 and is also referred to as 'Spy0416' (M1), 'SpyM3_0298' (M3), 'SpyM18_0464' (M18) and 'prtS'. GAS 057 has also been identified as a putative cell envelope proteinase. Amino acid and polynucleotide sequences of GAS 057 of an M1 strain are set forth in the sequence listing as SEQ ID NOS: 116 and 117.

25 Preferred GAS 057 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 116; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 116, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 057 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 116. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 116. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 116. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 116 (shown below) is removed. (SEQ ID NO: 118 comprises the underlined N-terminal leader sequence. SEQ ID NO: 119 comprises a fragment of GAS 57 where the N-terminal leader sequence is removed.) In another example, the underlined amino acid sequence at the C-terminus of SEQ ID NO: 116 is removed. (SEQ ID NO: 120 comprises the underlined C-terminal hydrophobic region. SEQ ID NO: 121 comprises a fragment of GAS 57 where the C-terminal hydrophobic

region is removed. SEQ ID NO: 122 comprises a fragment of GAS 57 where both the N-terminal leader sequence and the C-terminal hydrophobic region are removed.) Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

5 **SEQ ID NO: 116**

MEKKQRFSLRKYKSGTFSVLIGSVFLVMTTVAADELSTMSEPTITNHAQQQAQHLTNTLSSAESKSQDTSQITLKTNR
 EKEQSQDLVSEPTTTELADTDAASMAN TGSDATQKSASLPPVNTDVHDWVKTKGAWDKGYKGQGVVAVIDTGIDPAHQ
 10 MRI SDVSTAKVKSKE DMLARQKAAGIN YGSWINDKVVFAHNYVENS DN I KENQFEDFDEDWENFEFDAEAE PKAIKKHKI
 YRPQSTQAPKETVIKTEETD GSHDIDWTQTDDDTKYESHGMHVTGIVAGNSKEAAATGERFLGIAPEAQVMFMRV FANDI
 MGS A E S L F I K A I E D A V A L G A D V I N L S L G T A N G A Q L S G S K P L M E A I E K A K K A G V S V V V A A G N E R V Y G S D H D D P L A T N P D Y G
 L V G S P S T G R T P T S V A A I N S K W V I Q R L M T V K E L E N R A D L N H G K A I Y S E S V D F K D I K D S L G Y D K S H Q F A Y V K E S T D A G Y N A Q
 D V K G K I A L I E R D P N K T Y D E M I A L A K K H G A L G V L I F N N K P G Q S N R S M R L T A N G M G I P S A F I S H E F G K A M S Q L N G N G T G S L E
 F D S V V S K A P S Q K G N E M N H F S N W G L T S D G Y L K P D I T A P G G D I Y S T Y N D N H Y G S Q T G T S M A S P Q I A G A S L L V K Q Y L E K T Q P N
 15 L P K E K I A D I V K N L L M S N A Q I H V N P E T K T T T S P R Q Q G A G L L N I D G A V T S G L Y V T G K D N Y G S I S L G N I T D T M T F D V T V H N L S
 N K D K T L R Y D T E L L T D H V D P Q K G R F T L T S H S L K T Y Q G G E V T V P A N G K V T V R V T M D V S Q F T K E L T K Q M P N G Y Y L E G F V R F R D
 S Q D D Q L N R V N I P F V G F K G Q F E N L A V A E E S I Y R L K S Q G K T G F Y F D E S G P K D D I Y V G K H F T G L V T L G S E T N V S T K T I S D N G L
 H T L G T F K N A D G K F I L E K N A Q G N P V L A I S P N G D N N Q D F A A F K G V F L R K Y Q G L K A S V Y H A S D K E H K N P L W V S P E S F K G D K N F
 N S D I R F A K S T L L L G T A F S G K S L T G A E L P D G H Y H Y V V S Y Y P D V V G A K R Q E M T F D M I L D R Q K P V L S Q A T F D P E T N R F K P E P L
 K D R G L A G V R K D S V F Y L E R K D N K P Y T V T I N D S Y K Y V S V E D N K T F V E R Q A D G S F I L P L D K A K L G D F Y M V E D F A G N V A I A K L
 20 G D H L P Q T L G K T P I K L K L T D G N Y Q T K E T L K D N L E M T Q S D T G L V T N Q A Q L A V V H R N Q P Q S Q L T K M N Q D F F I S P N E D G N K D F V
 A F K G L K N N V Y N D L T V N V Y A K D D H Q K Q T P I W S S Q A G A S V S A I E S T A W Y G I T A R G S K V M P G D Y Q Y V V T Y R D E H G K E H Q K Q Y T
 I S V N D K K P M I T Q G R F D T I N G V D H F T P D K T K A L D S S G I V R E E V F Y L A K K N G R K F D V T E G K D G I T V S D N K V Y I P K N P D G S Y T
 I S K R D G V T L S D Y Y L V E D R A G N V S F A T L R D L K A V G K D K A V V N F G L D L P V P E D K Q I V N F T Y L V R D A D G K P I E N L E Y Y N N S G
 N S L I L P Y G K Y T V E L L T Y D T N A A K L E S D K I V S F T L S A D N N F Q Q V T F K I T M L A T S Q I T A H F D H L L P E G S R V S L K T A Q D Q L I P
 25 L E Q S L Y V P K A Y G K T V Q E G T Y E V V V S L P K G Y R I E G N T K V N T L P N E V H E L S L R L V K V G D A S D S T G D H K V M S K N N S Q A L T A S A
 T P T K S T T S A T A K A L P S T G E K M G L K L R I V G L V L L G L T C V F S R K K S T K D

Representative examples of immunization with GAS antigens of the invention in the murine mouse model discussed above are summarized in Figure 8. The first column identifies the GAS antigen used in the experiment. In some instances purification aspects are referenced in this list. Also, modifications to the polynucleotide sequence which have been made to facilitate the recombinant expression of the antigen are denoted in the chart with the following annotations: “a” indicates that N or C terminal hydrophobic regions have been removed; RR indicates codon optimisation; “NH” and “CH” correspond to the expression vectors similar to those indicated in the GAS 40 construct examples. Where a p value is given, it was calculated based on the control HIS stop values at the bottom of the chart.

Mice immunized with GAS 40 yielded substantially improved survival rates on challenge – in a collection of over 100 mice immunizations, immunization with GAS 40 yielded over 50% survival. The other GAS antigens in the chart offered an amount of protection that, for example if combined with GAS 40, could offer improved protection.

The immunogenicity of other known GAS antigens may be improved by combination with two or more GAS the first antigen group. Such other known GAS antigens include a second antigen group consisting of (1) one or more variants of the M surface protein or fragments thereof, (2) fibronectin-binding protein, (3) streptococcal heme-associated protein, or (4) SagA. These antigens are referred to herein as the “second antigen group”.

The invention thus includes an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group and one, two, three, or four GAS antigens of the second antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Still more preferably, the

combination consists of three, four or five GAS antigens from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination of GAS antigens includes one or more variants of the M surface protein.

Each of the GAS antigens of the second antigen group are described in more detail below.

5 (1) *M surface protein*

The M protein is a GAS virulence factor which has been associated with both colonization and resistance to phagocytosis. Over 100 different type variants of the M protein have been identified on the basis of antigenic specificity and M protein is thought to be the major cause of antigenic shift and antigenic drift in GAS. The M protein also binds fibrinogen from serum and blocks the binding of complement to the underlying peptidoglycan. This action is thought to increase GAS survival within a mammalian host by
10 inhibiting phagocytosis.

Unfortunately, the GAS M protein contains some epitopes which mimic those of mammalian muscle and connective tissue. Certain GAS M proteins may be rheumatogenic since they contain epitopes related to heart muscle, and may lead to autoimmune rheumatic carditis (rheumatic fever) following an acute infection.

15 Epitopes having increased bactericidal activity and having decreased likelihood of cross-reacting with human tissues have been identified in the amino terminal region and combined into fusion proteins containing approximately six, seven, or eight M protein fragments linked in tandem. See Hu et al., *Infection & Immunity* (2002) 70(4):2171 – 2177; Dale, *Vaccine* (1999) 17:193 – 200; Dale et al., *Vaccine* 14(10):944 – 948; WO 02/094851 and WO 94/06465. (Each of the M protein variants, fragments
20 and fusion proteins described in these references are specifically incorporated herein by reference.)

Accordingly, the compositions of the invention may further comprise a GAS M surface protein or a fragment or derivative thereof. One or more GAS M surface protein fragments may be combined together in a fusion protein. Alternatively, one or more GAS M surface protein fragments are combined with a GAS antigen or fragment thereof of the first antigen group. One example of a GAS M protein is set forth in the
25 sequence listing as SEQ ID NO: 123.

Preferred GAS M proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to a known M protein such as SEQ ID NO: 123; and/or (b) which is a fragment
30 of at least *n* consecutive amino acids of a known M protein such as SEQ ID NO: 123, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS M proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 123. Preferred fragments of (b) comprise an epitope from a known M protein, such as SEQ ID NO: 123. Preferably, the fragment is one of those described in the references above. Preferably, the fragment is constructed in a fusion protein with one or more additional M protein fragments. Other preferred fragments
35 lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of a known M protein such as SEQ ID NO: 123. Other fragments omit one or more domains of the protein (*e.g.*

omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(2) *Fibronectin-binding protein*

GAS fibronectin-binding protein ('SfbI') is a multifunctional bacterial protein thought to mediate attachment of the bacteria to host cells, facilitate bacterial internalization into cells and to bind to the Fc fragment of human IgG, thus interfering with Fc-receptor mediated phagocytosis and antibody-dependent cell cytotoxicity. Immunization of mice with SfbI and an 'H12 fragment' (encoded by positions 1240 – 1854 of the SfbI gene) are discussed in Schulze et al., Vaccine (2003) 21:1958 – 1964; Schulze et al., Infection and Immunity (2001) 69(1):622 – 625 and Guzman et al., Journal of Infectious Diseases (1999) 179:901 – 906. One example of an amino acid sequence for GAS SfbI is shown in the sequence listing as SEQ ID NO: 124.

Preferred SfbI proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 124; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 124, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These SfbI proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 124. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 124. Preferably, the fragment is one of those described in the references above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 124. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(3) *Streptococcal heme-associated protein*

The GAS streptococcal heme-associated protein ('Shp') has been identified as a GAS cell surface protein. It is thought to be cotranscribed with genes encoding homologues of an ABC transporter involved in iron uptake in gram-negative bacteria. The Shp protein is further described in Lei et al., "Identification and Characterization of a Novel Heme-Associated Cell Surface Protein Made by *Streptococcus pyogenes*", Infection and Immunity (2002) 70(8):4494 – 4500. One example of a Shp protein is shown in the sequence listing as SEQ ID NO: 125.

Preferred Shp proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 125; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 125, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These Shp proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 125. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 125. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 125. Other fragments omit one or more domains of the protein

(e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(4) *SagA*

Streptolysin S (SLS), also known as 'SagA', is thought to be produced by almost all GAS colonies. This cytolytic toxin is responsible for the beta-hemolysis surrounding colonies of GAS grown on blood agar and is thought to be associated with virulence. While the full SagA peptide has not been shown to be immunogenic, a fragment of amino acids 10 – 30 (SagA 10 – 30) has been used to produce neutralizing antibodies. See Dale et al., "Antibodies against a Synthetic Peptide of SagA Neutralize the Cytolytic Activity of Streptolysin S from Group A Streptococci", *Infection and Immunity* (2002) 70(4):2166 – 2170. The amino acid sequence of SagA 10 – 30 is shown in the sequence listing as SEQ ID NO: 126.

Preferred SagA 10-30 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 126; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 126, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, or 20). These SagA 10 - 30 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 126.

There is an upper limit to the number of GAS antigens which will be in the compositions of the invention. Preferably, the number of GAS antigens in a composition of the invention is less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, or less than 3. Still more preferably, the number of GAS antigens in a composition of the invention is less than 6, less than 5, or less than 4. Still more preferably, the number of GAS antigens in a composition of the invention is 3. The GAS antigens used in the invention are preferably isolated, i.e., separate and discrete, from the whole organism with which the molecule is found in nature or, when the polynucleotide or polypeptide is not found in nature, is sufficiently free of other biological macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose.

Fusion proteins

The GAS antigens used in the invention may be present in the composition as individual separate polypeptides, but it is preferred that at least two (i.e. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) of the antigens are expressed as a single polypeptide chain (a 'hybrid' polypeptide). Hybrid polypeptides offer two principal advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

The hybrid polypeptide may comprise two or more polypeptide sequences from the first antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a GAS

antigen or a fragment thereof of the first antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise different epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the first antigen group and one or more polypeptide sequences from the second antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a GAS antigen or a fragment thereof from the first antigen group and said second amino acid sequence selected from a GAS antigen or a fragment thereof from the second antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise different epitopes.

Hybrids consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten GAS antigens are preferred. In particular, hybrids consisting of amino acid sequences from two, three, four, or five GAS antigens are preferred.

Different hybrid polypeptides may be mixed together in a single formulation. Within such combinations, a GAS antigen may be present in more than one hybrid polypeptide and/or as a non-hybrid polypeptide. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Hybrid polypeptides can be represented by the formula $\text{NH}_2\text{-A-}\{-\text{X-L-}\}_n\text{-B-COOH}$, wherein: X is an amino acid sequence of a GAS antigen or a fragment thereof from the first antigen group or the second antigen group; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and n is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of $X_2 \dots X_n$ will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

For each n instances of $\{-\text{X-L-}\}$, linker amino acid sequence -L- may be present or absent. For instance, when $n=2$ the hybrid may be $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-L}_2\text{-COOH}$, $\text{NH}_2\text{-X}_1\text{-X}_2\text{-COOH}$, $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-COOH}$, $\text{NH}_2\text{-X}_1\text{-X}_2\text{-L}_2\text{-COOH}$, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_n where $n = 2, 3, 4, 5, 6, 7, 8, 9, 10$ or more), and histidine tags (*i.e.* His_n where $n = 3, 4, 5, 6, 7, 8, 9, 10$ or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG, with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(\text{Gly})_4$ tetrapeptide being a typical poly-glycine linker.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein

trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_{*n*}, where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X₁ lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

5 -B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_{*n*}, where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Most preferably, *n* is 2 or 3.

The fusion constructs of the invention may include a combination of two or more GAS antigens, wherein said combination includes GAS 40 or a fragment thereof or a polypeptide having sequence identity thereto.

15 The fusion constructs of the invention may include a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group, said first antigen group consisting of: GAS 117, GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS 384, GAS 202, and GAS 057. Preferably, the combination of GAS antigens consists of three, four, five, six, seven, eight, nine, or ten GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens consists of three, four, or five GAS antigens selected from the first antigen group.

20 GAS 39, GAS 40, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511 are particularly preferred GAS antigens for use in the fusion constructs of the invention. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination includes GAS 40.

Recombinant expression of the fusion constructs of the invention may be improved or optimised by the same methods described for the expression of the GAS antigens alone (discussed above). Fusion constructs of GAS 40 and GAS 117 are exemplified below.

30 In the first example, GAS 117 is linked to GAS 40a-RR. (As discussed above, GAS 40a-RR is a codon optimised GAS 40 sequence where the N-terminal leader sequence and the C-terminal transmembrane sequence are removed). In this construct a GAS 117 fragment (where the N-terminal leader sequence is removed) is placed to the N-terminus of the GAS 40 sequence and a HIS tag is added to the C-terminus of the GAS 40 sequence. This construct is designated "117-40a-RR". Amino acid and polynucleotide sequences for this construct are shown in the sequence listing as SEQ ID NOS: 127 and 128.

35 The GAS 117 and GAS 40 sequences are preferably linked by a linker sequence comprising multiple Glycine residues. For example, the linker used in 117-40a-RR fusion construct, a linker sequence of SEQ ID NO: 129 (YASGGGS) is used.

In a second example, the relative locations of the GAS 40 and GAS 117 sequences can be exchanged. In this construct, designated "40a-RR-117", the GAS 40a-RR sequence is placed to the N-terminus of the GAS 117 sequence and the HIS tag is added to the C-terminus of the GAS 117 sequence. Amino acid and polynucleotide sequences for this fusion construct are shown in the sequence listing as SEQ ID NOS: 130 and 131.

Alternatively, the fusion constructs may be designed without codon optimisations. For example, polynucleotide and amino acid sequences for fusion construct "117-40a" is shown in the sequence listing as SEQ ID NOS: 132 and 133. (While no codon optimisations were used, three point mutations apparently occurred during the cloning, only one of which involved a conservative amino acid change (Glucine to Glycine). In the murine immunization model (previously discussed above), immunization with "117-40a" has yielded up to 80 % survival upon challenge.

A preferred GAS40 fusion sequence comprises a fragment of GAS 40 comprising one or more of the coiled-coil regions. For example, the fusion construct may comprise a GAS 40 sequence comprising the first coiled-coil region. "117-40N" is an example of this type of construct. Amino acid and polynucleotide sequences for this construct are shown in the sequence listing as SEQ ID NOS; 132 and 133.

The invention also provides nucleic acids encoding hybrid polypeptides of the invention. Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (*e.g.* 65°C in a 0.1xSSC, 0.5% SDS solution).

The GAS antigens of the invention may also be used to prepare antibodies specific to the GAS antigens. The antibodies are preferably specific to the first or second coiled-coil regions of GAS 40. The invention also includes the use of combination of two or more types of antibodies selected from the group consisting of antibodies specific to GBS 80, GAS 117, GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS 384, GAS 202, and GAS 057. Preferably, the combination includes an antibody specific to GAS 40, or a fragment thereof.

The GAS specific antibodies of the invention include one or more biological moieties that, through chemical or physical means, can bind to or associate with an epitope of a GAS polypeptide. The antibodies of the invention include antibodies which specifically bind to a GAS antigen, preferably GAS 80. The invention includes antibodies obtained from both polyclonal and monoclonal preparations, as well as the following: hybrid (chimeric) antibody molecules (see, for example, Winter *et al.* (1991) *Nature* 349: 293-299; and US Patent No. 4,816,567; F(ab')₂ and F(ab) fragments; F_v molecules (non-covalent heterodimers, see, for example, Inbar *et al.* (1972) *Proc Natl Acad Sci USA* 69:2659-2662; and Ehrlich *et al.* (1980) *Biochem* 19:4091-4096); single-chain F_v molecules (sFv) (see, for example, Huston *et al.* (1988) *Proc Natl Acad Sci USA* 85:5897-5883); dimeric and trimeric antibody fragment constructs; minibodies (see, *e.g.*, Pack *et al.* (1992) *Biochem* 31:1579-1584; Cumber *et al.* (1992) *J Immunology* 149B: 120-126); humanized antibody molecules (see, for example, Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeyan *et al.* (1988) *Science* 239:1534-1536; and U.K. Patent Publication No. GB 2,276,169, published 21 September

1994); and, any functional fragments obtained from such molecules, wherein such fragments retain immunological binding properties of the parent antibody molecule. The invention further includes antibodies obtained through non-conventional processes, such as phage display.

Preferably, the GAS specific antibodies of the invention are monoclonal antibodies. Monoclonal antibodies of the invention include an antibody composition having a homogeneous antibody population. Monoclonal antibodies of the invention may be obtained from murine hybridomas, as well as human monoclonal antibodies obtained using human rather than murine hybridomas. See, *e.g.*, Cote, *et al. Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, 1985, p 77.

Polypeptides of the invention can be prepared by various means (*e.g.* recombinant expression, purification from cell culture, chemical synthesis, *etc.*) and in various forms (*e.g.* native, fusions, non-glycosylated, lipidated, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other GAS or host cell proteins).

Nucleic acid according to the invention can be prepared in many ways (*e.g.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself, *etc.*) and can take various forms (*e.g.* single stranded, double stranded, vectors, probes, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other GAS or host cell nucleic acids).

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (*e.g.* phosphorothioates, *etc.*), and also peptide nucleic acids (PNA), *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (*e.g.* for antisense or probing purposes).

The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means.

The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (*e.g.* PCR).

The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

Strains

Preferred polypeptides of the invention comprise an amino acid sequence found in an M1, M3 or M18 strain of GAS. The genomic sequence of an M1 GAS strain is reported at Ferretti et al, PNAS (2001) 98(8):4658 – 4663. The genomic sequence of an M3 GAS strain is reported at Beres et al., PNAS (2002) 99(15):10078 – 10083. The genomic sequence of an M18 GAS strain is reported at Smooet et al., PNAS (2002) 99(7):4668 – 4673.

Where hybrid polypeptides are used, the individual antigens within the hybrid (*i.e.* individual -X-moieties) may be from one or more strains. Where $n=2$, for instance, X_2 may be from the same strain as X_1

or from a different strain. Where $n=3$, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2 \neq X_3$ (iii) $X_1 \neq X_2=X_3$ (iv) $X_1 \neq X_2 \neq X_3$ or (v) $X_1=X_3 \neq X_2$, etc.

Purification and Recombinant Expression

The GAS antigens of the invention may be isolated from a *Streptococcus pyogenes*, or they may be recombinantly produced, for instance, in a heterologous host. Preferably, the GAS antigens are prepared using a heterologous host. The heterologous host may be prokaryotic (e.g. a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium*, *Neisseria lactamica*, *Neisseria cinerea*, *Mycobacteria* (e.g. *M.tuberculosis*), yeasts, etc.

Recombinant production of polypeptides is facilitated by adding a tag protein to the GAS antigen to be expressed as a fusion protein comprising the tag protein and the GAS antigen. Such tag proteins can facilitate purification, detection and stability of the expressed protein. Tag proteins suitable for use in the invention include a polyarginine tag (Arg-tag), polyhistidine tag (His-tag), FLAG-tag, Strep-tag, c-myc-tag, S-tag, calmodulin-binding peptide, cellulose-binding domain, SBP-tag, chitin-binding domain, glutathione S-transferase-tag (GST), maltose-binding protein, transcription termination anti-terminiation factor (NusA), *E. coli* thioredoxin (TrxA) and protein disulfide isomerase I (DsbA). Preferred tag proteins include His-tag and GST. A full discussion on the use of tag proteins can be found at Terpe et al., Appl Microbiol Biotechnol (2003) 60:523 – 533.

After purification, the tag proteins may optionally be removed from the expressed fusion protein, i.e., by specifically tailored enzymatic treatments known in the art. Commonly used proteases include enterokinase, tobacco etch virus (TEV), thrombin, and factor X_a .

Immunogenic compositions and medicaments

Compositions of the invention are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 8, preferably about 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen-free. The composition may be isotonic with respect to humans.

Vaccines according to the invention may either be prophylactic (i.e. to prevent infection) or therapeutic (i.e. to treat infection), but will typically be prophylactic. Accordingly, the invention includes a method for the therapeutic or prophylactic treatment of a *Streptococcus pyogenes* infection in an animal susceptible to streptococcal infection comprising administering to said animal a therapeutic or prophylactic amount of the immunogenic compositions of the invention. Preferably, the immunogenic composition comprises a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group. Preferably, the combination of GAS antigens consists of three, four, five, six, seven, eight, nine, or ten GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens consists of three, four, or five GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117.

Alternatively, the invention includes an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group and

one, two, three, or four GAS antigens of the second antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Still more preferably, the combination consists of three, four or five GAS antigens from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination of GAS antigens includes one or more variants of the M surface protein.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of the compositions of the invention in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine. The invention also provides for a kit comprising a first component comprising a combination of GAS antigens. In one embodiment, the combination of GAS antigens consists of a mixture of two to thirty-one GAS antigens selected from the first antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Preferably, the combination consists of three, four, or five GAS antigens from the first antigen group. Preferably, the combination includes either or both of GAS 117 and GAS 040.

In another embodiment, the kit comprises a first component comprising a combination of GAS antigens consisting of a mixture of two to thirty-one GAS antigens of the first antigen group and one, two, three, or four GAS antigens of the second antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Still more preferably, the combination consists of three, four or five GAS antigens from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination of GAS antigens includes one or more variants of the M surface protein.

The invention also provides a delivery device pre-filled with the immunogenic compositions of the invention.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies and/or cell-mediated immunity. The method may raise a booster response.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably a teenager or an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

These uses and methods are preferably for the prevention and/or treatment of a disease caused by *Streptococcus pyogenes* (*e.g.* pharyngitis (such as streptococcal sore throat), scarlet fever, impetigo, erysipelas, cellulitis, septicemia, toxic shock syndrome, necrotizing fasciitis (flesh eating disease) and sequelae (such as rheumatic fever and acute glomerulonephritis)). The compositions may also be effective against other streptococcal bacteria.

One way of checking efficacy of therapeutic treatment involves monitoring GAS infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against the GAS antigens in the compositions of the invention after administration of the composition.

5 Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral (*e.g.* tablet, spray), vaginal, topical, transdermal (*e.g.* see WO99/27961) or transcutaneous (*e.g.* see WO02/074244 and WO02/064162), intranasal (*e.g.* see WO03/028760), ocular, aural, pulmonary or other mucosal administration.

10 The invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes *e.g.* a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, *etc.*

15 The compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup
20 (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to administration to a patient. Such kits may comprise one or more antigens in liquid form and one or more lyophilised antigens.
25 Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate,
30 primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Further components of the composition

35 The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids,

polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in Gennaro (2000) *Remington: The Science and Practice of Pharmacy*. 20th ed., ISBN: 0683306472.

Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant.

Preferred further adjuvants include, but are not limited to, one or more of the following set forth below:

A. Mineral Containing Compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulphates, *etc.* (*e.g.* see chapters 8 & 9 of *Vaccine design: the subunit and adjuvant approach* (1995) Powell & Newman. ISBN 0-306-44867-X}), or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt. See WO00/23105.

B. Oil-Emulsions

Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See WO90/14837. See also, Podda, "The adjuvanted influenza vaccines with novel adjuvants: experience with the MF59-adjuvanted vaccine", *Vaccine* (2001) 19: 2673-2680; Frey et al., "Comparison of the safety, tolerability, and immunogenicity of a MF59-adjuvanted influenza vaccine and a non-adjuvanted influenza vaccine in non-elderly adults", *Vaccine* (2003) 21:4234-4237. MF59 is used as the adjuvant in the FLUAD™ influenza virus trivalent subunit vaccine.

Particularly preferred adjuvants for use in the compositions are submicron oil-in-water emulsions. Preferred submicron oil-in-water emulsions for use herein are squalene/water emulsions optionally containing varying amounts of MTP-PE, such as a submicron oil-in-water emulsion containing 4-5% w/v squalene, 0.25-1.0% w/v Tween 80™ (polyoxyethylsorbitan monooleate), and/or 0.25-1.0% Span 85™ (sorbitan trioleate), and, optionally, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), for example, the submicron oil-in-water emulsion known as "MF59" (International Publication No. WO90/14837; US Patent Nos. 6,299,884 and 6,451,325, incorporated herein by reference in their entireties; and Ott et al., "MF59 -- Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines" in *Vaccine Design: The Subunit and Adjuvant Approach* (Powell, M.F. and Newman, M.J. eds.) Plenum Press, New York, 1995, pp. 277-296). MF59 contains 4-5% w/v Squalene (*e.g.* 4.3%), 0.25-0.5% w/v Tween 80™, and 0.5% w/v Span 85™ and optionally contains various amounts of MTP-PE, formulated into submicron particles using a microfluidizer

such as Model 110Y microfluidizer (Microfluidics, Newton, MA). For example, MTP-PE may be present in an amount of about 0-500 µg/dose, more preferably 0-250 µg/dose and most preferably, 0-100 µg/dose. As used herein, the term "MF59-0" refers to the above submicron oil-in-water emulsion lacking MTP-PE, while the term MF59-MTP denotes a formulation that contains MTP-PE. For instance, "MF59-100" contains 100 µg MTP-PE per dose, and so on. MF69, another submicron oil-in-water emulsion for use herein, contains 4.3% w/v squalene, 0.25% w/v Tween 80™, and 0.75% w/v Span 85™ and optionally MTP-PE. Yet another submicron oil-in-water emulsion is MF75, also known as SAF, containing 10% squalene, 0.4% Tween 80™, 5% pluronic-blocked polymer L121, and thr-MDP, also microfluidized into a submicron emulsion. MF75-MTP denotes an MF75 formulation that includes MTP, such as from 100-400 µg MTP-PE per dose.

Submicron oil-in-water emulsions, methods of making the same and immunostimulating agents, such as muramyl peptides, for use in the compositions, are described in detail in International Publication No. WO90/14837 and US Patent Nos. 6,299,884 and 6,451,325, incorporated herein by reference in their entireties.

Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

C. Saponin Formulations

Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsapilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. Saponin compositions have been purified using High Performance Thin Layer Chromatography (HP-LC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in U.S. Patent No. 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO 96/33739).

Combinations of saponins and cholesterol can be used to form unique particles called Immunostimulating Complexes (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP 0 109 942, WO 96/11711 and WO 96/33739. Optionally, the ISCOMS may be devoid of additional detergent. See WO00/07621.

A review of the development of saponin based adjuvants can be found at Barr, et al., Advanced Drug Delivery Reviews (1998) 32:247 – 271. See also Sjolander, et al., Advanced Drug Delivery Reviews (1998) 32:321 – 338.

C. Virosomes and Virus Like Particles (VLPs)

Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses.

5 These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Q β -phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO 03/024480, WO 03/024481, and Niikura et al., *Virology* (2002) 293:273 – 280, Lenz et al., *Journal of Immunology* (2001) 5246 – 5355; Pinto, et al., *Journal of Infectious Diseases* (2003) 188:327 – 338 and Gerber et al., *Journal of Virology* (2001) 75(10):4752 – 4760. Virosomes are discussed further in, for example, Gluck et al., *Vaccine* (2002) 20:B10 – B16.

D. Bacterial or Microbial Derivatives

15 Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

(1) *Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)*

Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL).

3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred “small particle” form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such “small particles” of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529. See Johnson *et al.* (1999) *Bioorg Med Chem Lett* 9:2273-2278.

(2) *Lipid A Derivatives*

25 Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in Meraldi et al., *Vaccine* (2003) 21:2485 – 2491 and Pajak, et al., *Vaccine* (2003) 21:836 – 842.

(3) *Immunostimulatory oligonucleotides*

30 Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogues such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be replaced with an analogue such as 2'-deoxy-7-deazaguanosine. See Kandimalla, et al., *Nucleic Acids Research* (2003) 31(9): 2393 – 2400; WO 02/26757 and WO 99/62923 for examples of possible analogue substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Krieg, *Nature Medicine* (2003) 9(7): 831 – 835; McCluskie,

et al., FEMS Immunology and Medical Microbiology (2002) 32:179 – 185; WO 98/40100, U.S. Patent No. 6,207,646, U.S. Patent No. 6,239,116, and U.S. Patent No. 6,429,199.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See Kandimalla, et al., Biochemical Society Transactions (2003) 31 (part 3): 654 – 658. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such as a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in Blackwell, et al., J. Immunol. (2003) 170(8):4061 – 4068; Krieg, TRENDS in Immunology (2002) 23(2): 64 – 65 and WO 01/95935. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form “immunomers”. See, for example, Kandimalla, et al., BBRC (2003) 306:948 – 953; Kandimalla, et al., Biochemical Society Transactions (2003) 31(part 3):664 – 658; Bhagat et al., BBRC (2003) 300:853 – 861 and WO 03/035836.

(4) *ADP-ribosylating toxins and detoxified derivatives thereof.*

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (i.e., *E. coli* heat labile enterotoxin “LT), cholera (“CT”), or pertussis (“PT”). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO 95/17211 and as parenteral adjuvants in WO 98/42375. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63.

E. Human Immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon- γ), macrophage colony stimulating factor, and tumor necrosis factor.

F. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres (Singh *et al.* (2001) *J. Cont. Rele.* 70:267-276) or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrrolidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. *E.g.*, WO99/27960.

G. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150 μ m in diameter, more preferably ~200nm to ~30 μ m in diameter, and most preferably ~500nm to ~10 μ m in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α -hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

H. Liposomes

Examples of liposome formulations suitable for use as adjuvants are described in U.S. Patent No. 6,090,406, U.S. Patent No. 5,916,588, and EP 0 626 169.

I. Polyoxyethylene ether and Polyoxyethylene Ester Formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. WO99/52549. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (WO01/21207) as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (WO01/21152).

Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

J. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in Andrianov et al., "Preparation of hydrogel microspheres by coacervation of aqueous polyphosphazene solutions", *Biomaterials* (1998) 19(1 – 3):109 – 115 and Payne et al., "Protein Release from Polyphosphazene Matrices", *Adv. Drug. Delivery Review* (1998) 31(3):185 – 196.

K. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetylmuramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use as adjuvants in the invention include Imiquimod and its homologues, described further in Stanley, "Imiquimod and the imidazoquinolones: mechanism of action and therapeutic potential" *Clin Exp Dermatol* (2002) 27(7):571 – 577 and Jones, "Resiquimod 3M", *Curr Opin Investig Drugs* (2003) 4(2):214 – 218.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

- (1) a saponin and an oil-in-water emulsion (WO99/11241);
- (2) a saponin (e.g., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) (see WO 94/00153);
- (3) a saponin (e.g., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) + a cholesterol;
- (4) a saponin (e.g. QS21) + 3dMPL + IL-12 (optionally + a sterol) (WO98/57659);
- (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (European patent applications 0835318, 0735898 and 0761231);
- (6) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-

MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(7) RibTM adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DetoxTM); and

(8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

(9) one or more mineral salts (such as an aluminum salt) + an immunostimulatory oligonucleotide (such as a nucleotide sequence including a CpG motif).

Aluminium salts and MF59 are preferred adjuvants for parenteral immunisation. Mutant bacterial toxins are preferred mucosal adjuvants.

The composition may include an antibiotic.

Further antigens

The compositions of the invention may further comprise one or more additional non-GAS antigens, including additional bacterial, viral or parasitic antigens.

In one embodiment, the GAS antigen combinations of the invention are combined with one or more additional, non-GAS antigens suitable for use in a paediatric vaccine. For example, the GAS antigen combinations may be combined with one or more antigens derived from a bacteria or virus selected from the group consisting of *N. meningitidis* (including serogroup A, B, C, W135 and/or Y), *Streptococcus pneumoniae*, *Bordetella pertussis*, *Moraxella catarrhalis*, *Tetanus*, *Diphtheria*, Respiratory Syncytial virus ('RSV'), polio, measles, mumps, rubella, and rotavirus.

In another embodiment, the GAS antigen combinations of the invention are combined with one or more additional, non-GAS antigens suitable for use in a vaccine designed to protect elderly or immunocompromised individuals. For example, the GAS antigen combinations may be combined with an antigen derived from the group consisting of *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Listeria monocytogenes*, influenza, and Parainfluenza virus ('PIV').

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity {e.g. Ramsay *et al.* (2001) *Lancet* 357(9251):195-196; Lindberg (1999) *Vaccine* 17 Suppl 2:S28-36; Buttery & Moxon (2000) *J R Coll Physicians Lond* 34:163-168; Ahmad & Chapnick (1999) *Infect Dis Clin North Am* 13:113-133, vii. Goldblatt (1998) *J. Med. Microbiol.* 47:563-567; European patent 0 477 508; US Patent No. 5,306,492; WO98/42721; *Conjugate Vaccines* (eds. Cruse *et al.*) ISBN 3805549326, particularly vol. 10:48-114; Hermanson (1996) *Bioconjugate Techniques* ISBN: 0123423368 or 012342335X}. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM₁₉₇ diphtheria toxoid is particularly preferred {*Research Disclosure*, 453077 (Jan 2002)}. Other carrier polypeptides include the *N.meningitidis* outer membrane protein {EP-A-0372501}, synthetic peptides { EP-A-0378881 and EP-A-0427347}, heat shock proteins { WO93/17712 and WO94/03208}, pertussis proteins {WO98/58668 and EP-A-0471177}, protein D from *H.influenzae* {WO00/56360}, cytokines {WO91/01146}, lymphokines, hormones, growth factors, toxin A or B from *C.difficile* {WO00/61761}, iron-uptake proteins { WO01/72337}, *etc.* Where a mixture comprises capsular

saccharides from both serogroups A and C, it may be preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

5 Toxic protein antigens may be detoxified where necessary *e.g.* detoxification of pertussis toxin by chemical and/or genetic means.

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

10 Antigens in the composition will typically be present at a concentration of at least 1µg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used {*e.g.* Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648; Scott-Taylor & Dalgleish (2000) *Expert Opin Investig Drugs* 9:471-480; Apostolopoulos & Plebanski (2000) *Curr Opin Mol Ther* 2:441-447; Ilan (1999) *Curr Opin Mol Ther* 1:116-120; Dubensky *et al.* (2000) *Mol Med* 6:723-732; Robinson & Pertmer (2000) *Adv Virus Res* 55:1-74; Donnelly *et al.* (2000) *Am J Respir Crit Care Med* 162(4 Pt 2):S190-193; Davis (1999) *Mt. Sinai J. Med.* 66:84-90}. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

Definitions

The term “comprising” means “including” as well as “consisting” *e.g.* a composition “comprising” X may consist exclusively of X or may include something additional *e.g.* X + Y.

25 The term “about” in relation to a numerical value x means, for example, $x \pm 10\%$.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489. Similar sequence identity methods can be used to determine sequence homology between two polynucleotide sequences.

35 The following example demonstrates one way of preparing recombinant GAS antigens of the invention and testing their efficacy in a murine model.

EXAMPLE 1: Preparation of recombinant GAS antigens of the invention and Demonstration of Efficacy in Murine Model.

Recombinant GAS proteins corresponding to two or more of the GAS antigens of the first antigen group are expressed as follows.

1. Cloning of GAS antigens for expression in E. coli

The selected GAS antigens were cloned in such a way to obtain two different kinds of recombinant proteins: (1) proteins having an hexa-histidine tag at the carboxy-terminus (Gas-His) and (2) proteins having the hexa-histidine tag at the carboxy-terminus and GST at the amino-terminus (Gst-Gas-His). Type (1) proteins were obtained by cloning in a pET21b+vector (available from Novagen). The type (2) proteins were obtained by cloning in a pGEX-NNH vector. This cloning strategy allowed for the GAS genomic DNA to be used to amplify the selected genes by PCR, to perform a single restriction enzyme digestion of the PCR products and to clone then simultaneously into both vectors.

(a) *Construction of pGEX-NNH expression vectors*

Two couples of complementary oligodeoxyribonucleotides are synthesised using the DNA synthesiser ABI394 (Perkin Elmer) and reagents from Cruachem (Glasgow, Scotland). Equimolar amounts of the oligo pairs (50 ng each oligo) are annealed in T4 DNA ligase buffer (New England Biolabs) for 10 min in a final volume of 50 µl and then left to cool slowly at room temperature. With the described procedure the following DNA linkers are obtained:

gexNN linker

NdeI NheI XmaI EcoRI NcoI SalI XhoI SacI
 GATCCCATATGGCTAGCCCGGGGAATTCGTCCATGGAGTGAGTCGACTGACTCGAGTGATCGAGCTC
 GGTATACCGATCGGGCCCCCTTAAGCAGGTACCTCACTCAGCTGACTGAGCTCACTAGCTCGAG

NotI

CTGAGCGGCCGCATGAA
 GACTCGCCGGCGTACTTTCGA

gexNNH linker

HindIII NotI XhoI Hexa-Histidine
 TCGACAAGCTTGC GGCCGCACTCGAGCATCACCATCACCATCACTGAT
 GTTCGAACGCCGGCGTGAGCACGTAGAGGTAGTGGTAGTGACTATCGA

The plasmid pGEX-KG [K. L. Guan and J. E. Dixon, *Anal. Biochem.* **192**, 262 (1991)] is digested with BamHI and HindIII and 100 ng is ligated overnight at 16 °C to the linker gexNN with a molar ratio of 3:1 linker/plasmid using 200 units of T4 DNA ligase (New England Biolabs). After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NN plasmid, having the correct linker, is selected by means of restriction enzyme analysis and DNA sequencing.

The new plasmid pGEX-NN is digested with SalI and HindIII and ligated to the linker gexNNH. After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NNH plasmid, having the correct linker, is selected by means of restriction enzyme analysis and DNA sequencing.

(b) *Chromosomal DNA preparation*

GAS SF370 strain is grown in THY medium until OD₆₀₀ is 0.6-0.8. Bacteria are then centrifuged, suspended in TES buffer with lysozyme (10mg/ml) and mutanolysine (10U/µl) and incubated 1 hr at 37° C.

Following treatment of the bacterial suspension with RNAase, Proteinase K and 10% Sarcosyl/EDTA, protein extraction with saturated phenol and phenol/chloroform is carried out. The resulting supernatant is precipitated with Sodium Acetate/Ethanol and the extracted DNA is pelleted by centrifugation, suspended in Tris buffer and kept at -20° C.

5 (c) *Oligonucleotide design*

Synthetic oligonucleotide primers are designed on the basis of the coding sequence of each GAS antigen using the sequence of *Streptococcus pyogenes* SF370 M1 strain. Any predicted signal peptide is omitted, by deducing the 5' end amplification primer sequence immediately downstream from the predicted leader sequence. For most GAS antigens, the 5' tail of the primers (see Table 1, below) include only one
10 restriction enzyme recognition site (NdeI, or NheI, or SpeI depending on the gene's own restriction pattern); the 3' primer tails (see Table 1) include a XhoI or a NotI or a HindIII restriction site.

5' tails		3' tails	
NdeI	5' GTGCGTCATATG 3'	XhoI	5' GCGTCTCGAG 3'
NheI	5' GTGCGTGCTAGC 3'	NotI	5' ACTCGCTAGCGGCCGC 3'
SpeI	5' GTGCGTACTAGT 3'	HindIII	5' GCGTAAGCTT 3'

Table 1. Oligonucleotide tails of the primers used to amplify genes encoding selected GAS antigens.

As well as containing the restriction enzyme recognition sequences, the primers include nucleotides
15 which hybridize to the sequence to be amplified. The number of hybridizing nucleotides depends on the melting temperature of the primers which can be determined as described [(Breslauer et al., Proc. Nat. Acad. Sci. 83, 3746-50 (1986)]. The average melting temperature of the selected oligos is 50-55 °C for the hybridizing region alone and 65-75 °C for the whole oligos. Oligos can be purchased from MWG-Biotech S.p.A. (Firenze, Italy).

20 (d) *PCR amplification*

The standard PCR protocol is as follows: 50 ng genomic DNA are used as template in the presence of 0,2 µM each primer, 200 µM each dNTP, 1,5 mM MgCl₂, 1x PCR buffer minus Mg (Gibco-BRL), and 2 units of Taq DNA polymerase (Platinum Taq, Gibco-BRL) in a final volume of 100 µl. Each sample undergoes a double-step amplification: the first 5 cycles are performed using as the hybridizing temperature
25 of one of the oligos excluding the restriction enzyme tail, followed by 25 cycles performed according to the hybridization temperature of the whole length primers. The standard cycles are as follows:

one cycle:

denaturation : 94 °C, 2 min,

30 5 cycles:

denaturation: 94 °C, 30 seconds,
hybridization: 51 °C, 50 seconds,
elongation: 72 °C, 1 min or 2 min and 40 sec, }

35 25 cycles:

denaturation: 94 °C, 30 seconds,
hybridization: 70 °C, 50 seconds,
elongation: 72 °C, 1 min or 2 min and 40 sec, }

72 °C, 7 min,
4 °C

5 The elongation time is 1 min for GAS antigens encoded by ORFs shorter than 2000 bp, and 2 min and 40 seconds for ORFs longer than 2000 bp. The amplifications are performed using a Gene Amp PCR system 9600 (Perkin Elmer).

To check the amplification results, 4 µl of each PCR product is loaded onto 1-1.5 agarose gel and the size of amplified fragments compared with DNA molecular weight standards (DNA markers III or IX, Roche). The PCR products are loaded on agarose gel and after electrophoresis the right size bands are excised from the gel. The DNA is purified from the agarose using the Gel Extraction Kit (Qiagen) following the instruction of the manufacturer. The final elution volume of the DNA is 50 µl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8). One µl of each purified DNA is loaded onto agarose gel to evaluate the yield.

(e) *Digestion of PCR fragments*

15 One-two µg of purified PCR products are double digested overnight at 37 °C with the appropriate restriction enzymes (60 units of each enzyme) using the appropriate restriction buffer in 100 µl final volume. The restriction enzymes and the digestion buffers are from New England Biolabs. After purification of the digested DNA (PCR purification Kit, Qiagen) and elution with 30 µl TE, 1 µl is subjected to agarose gel electrophoresis to evaluate the yield in comparison to titrated molecular weight standards (DNA markers III or IX, Roche).

(f) *Digestion of the cloning vectors (pET21b+ and pGEX-NNH)*

25 10 µg of plasmid is double digested with 100 units of each restriction enzyme in 400 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37 °C. After electrophoresis on a 1% agarose gel, the band corresponding to the digested vector is purified from the gel using the Qiagen Qiaex II Gel Extraction Kit and the DNA was eluted with 50 µl TE. The DNA concentration is evaluated by measuring OD₂₆₀ of the sample.

(g) *Cloning of the PCR products*

30 Seventy five ng of the appropriately digested and purified vectors and the digested and purified fragments corresponding to each selected GAS antigen are ligated in final volumes of 10-20 µl with a molar ratio of 1:1 fragment/vector, using 400 units T4 DNA ligase (New England Biolabs) in the presence of the buffer supplied by the manufacturer. The reactions are incubated overnight at 16 °C.

35 Transformation of *E coli* BL21 (Novagen) and *E coli* BL21-DE3 (Novagen) electrocompetent cells is performed using pGEX-NNH ligations and pET21b+ ligations respectively. The transformation procedure is as follows: 1-2 µl the ligation reaction is mixed with 50 µl of ice cold competent cells, then the cells are poured in a gene pulser 0.1 cm electrode cuvette (Biorad). After pulsing the cells in a MicroPulser electroporator (Biorad) following the manufacturer instructions the cells are suspended in 0.95 ml of SOC medium and incubated for 45 min at 37 °C under shaking. 100 and 900 µl of cell suspensions are plated on separate plates of agar LB 100 µg/ml Ampicillin and the plates are incubated overnight at 37 °C. The screening of the transformants is done by PCR: randomly chosen transformants are picked and suspended in

30 µl of PCR reaction mix containing the PCR buffer, the 4 dNTPs, 1,5 mM MgCl₂, Taq polymerase and appropriate forward and reverse oligonucleotide primers that are able to hybridize upstream and downstream from the polylinker of pET21b+ or pGEX-NNH vectors. After 30 cycles of PCR, 5 µl of the resulting products are run on agarose gel electrophoresis in order to select for positive clones from which the expected PCR band is obtained. PCR positive clones are chosen on the basis of the correct size of the PCR product, as evaluated by comparison with appropriate molecular weight markers (DNA markers III or IX, Roche).

2. Protein expression

PCR positive colonies are inoculated in 3 ml LB 100 µg/ml Ampicillin and grown at 37 °C overnight. 70 µl of the overnight culture is inoculated in 2 ml LB/Amp and grown at 37 °C until OD₆₀₀ of the pET clones reached the 0,4-0,8 value or until OD₆₀₀ of the pGEX clones reached the 0,8-1 value. Protein expression is then induced by adding 1 mM IPTG (Isopropil β-D thio-galacto-piranoside) to the mini-cultures. After 3 hours incubation at 37 °C the final OD₆₀₀ is checked and the cultures are cooled on ice. After centrifugation of 0.5 ml culture, the cell pellet is suspended in 50 µl of protein Loading Sample Buffer (60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerin, 0.1% w/v Bromophenol Blue, 100 mM DTT) and incubated at 100 °C for 5 min. A volume of boiled sample corresponding to 0.1 OD₆₀₀ culture is analysed by SDS-PAGE and Coomassie Blue staining to verify the presence of induced protein band.

3. Purification of the recombinant proteins

Single colonies are inoculated in 25 ml LB 100 µg/ml Ampicillin and grown at 37 °C overnight. The overnight culture is inoculated in 500 ml LB/Amp and grown under shaking at 25 °C until OD₆₀₀ 0.4-0.7. Protein expression is then induced by adding 1 mM IPTG to the cultures. After 3.5 hours incubation at 25 °C the final OD₆₀₀ is checked and the cultures are cooled on ice. After centrifugation at 6000 rpm (JA10 rotor, Beckman), the cell pellet is processed for purification or frozen at -20° C.

(a) *Procedure for the purification of soluble His-tagged proteins from E.coli*

- (1) Transfer the pellets from -20°C to ice bath and reconstitute with 10 ml 50 mM NaHPO₄ buffer, 300 mM NaCl, pH 8,0, pass in 40-50 ml centrifugation tubes and break the cells as per the following outline.
- (2) Break the pellets in the French Press performing three passages with in-line washing.
- (3) Centrifuge at about 30-40000 x g per 15-20 min. If possible use rotor JA 25.50 (21000 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.)
- (4) Equilibrate the Poly-Prep columns with 1 ml Fast Flow Chelating Sepharose resin with 50 mM phosphate buffer, 300 mM NaCl, pH 8,0.
- (5) Store the centrifugation pellet at -20°C, and load the supernatant in the columns.
- (6) Collect the flow through.
- (7) Wash the columns with 10 ml (2 ml + 2 ml + 4 ml) 50 mM phosphate buffer, 300 mM NaCl, pH 8.0.
- (8) Wash again with 10 ml 20 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8.0.
- (9) Elute the proteins bound to the columns with 4.5 ml (1.5 ml + 1.5 ml + 1.5 ml) 250 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8,0 and collect the 3 corresponding fractions of ~1.5 ml each. Add to each tube 15 µl DTT 200 mM (final concentration 2 mM)

(10) Measure the protein concentration of the first two fractions with the Bradford method, collect a 10 μg aliquot of proteins from each sample and analyse by SDS-PAGE. (N.B.: should the sample be too diluted, load 21 μl + 7 μl loading buffer).

(11) Store the collected fractions at +4°C while waiting for the results of the SDS-PAGE analysis.

(12) For immunisation prepare 4-5 aliquots of 100 μg each in 0.5 ml in 40% glycerol. The dilution buffer is the above elution buffer, plus 2 mM DTT. Store the aliquots at -20°C until immunisation.

(b) *Purification of His-tagged proteins from Inclusion bodies*

Purifications are carried out essentially according the following protocol:

(1) Bacteria are collected from 500 ml cultures by centrifugation. If required store bacterial pellets at -20°C. For extraction, resuspend each bacterial pellet in 10 ml 50 mM TRIS-HCl buffer, pH 8,5 on an ice bath.

(2) Disrupt the resuspended bacteria with a French Press, performing two passages.

(3) Centrifuge at 35000 x g for 15 min and collect the pellets. Use a Beckman rotor JA 25.50 (21000 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.).

(4) Dissolve the centrifugation pellets with 50 mM TRIS-HCl, 1 mM TCEP {Tris(2-carboxyethyl)-phosphine hydrochloride, Pierce} , 6M guanidium chloride, pH 8.5. Stir for ~ 10 min. with a magnetic bar.

(5) Centrifuge as described above, and collect the supernatant.

(6) Prepare an adequate number of Poly-Prep (Bio-Rad) columns containing 1 ml of Fast Flow Chelating Sepharose (Pharmacia) saturated with Nichel according to manufacturer recommendations.. Wash the columns twice with 5 ml of H₂O and equilibrate with 50 mM TRIS-HCl, 1 mM TCEP, 6M guanidinium chloride, pH 8.5.

(7) Load the supernatants from step 5 onto the columns, and wash with 5 ml of 50 mM TRIS-Hcl buffer, 1 mM TCEP, 6M urea, pH 8.5

(8) Wash the columns with 10 ml of 20 mM imidazole, 50 mM TRIS-HCl , 6M urea, 1 mM TCEP, pH 8.5. Collect and set aside the first 5 ml for possible further controls.

(9) Elute the proteins bound to the columns with 4.5 ml of a buffer containing 250 mM imidazole, 50 mM TRIS-HCl, 6M urea, 1 mM TCEP, pH 8.5. Add the elution buffer in three 1.5 ml aliquots, and collect the corresponding 3 fractions. Add to each fraction 15 μl DTT (final concentration 2 mM).

(10) Measure eluted protein concentration with the Bradford method, and analyse aliquots of ca 10 μg of protein by SDS-PAGE.

(11) Store proteins at -20°C in 40% (v/v) glycerol, 50 mM TRIS-HCl, 2M urea, 0.5 M arginine, 2 mM DTT, 0.3 mM TCEP, 83.3 mM imidazole, pH 8.5.

(c) *Procedure for the purification of GST-fusion proteins from E.coli*

(1) Transfer the bacterial pellets from -20°C to an ice bath and suspend with 7,5 ml PBS, pH 7,4 to which a mixture of protease inhibitors (CØMPLETE™ - Boehringer Mannheim, 1 tablet every 25 ml of buffer) has been added.

(2) Transfer to 40-50 ml centrifugation tubes and sonicate according to the following procedure:

- a. Position the probe at about 0,5 cm from the bottom of the tube
- b. Block the tube with the clamp

c. Dip the tube in an ice bath

d. Set the sonicator as follows: Timer → Hold, Duty Cycle → 55, Out. Control → 6.

e. perform 5 cycles of 10 impulses at a time lapse of 1 minute (i.e. one cycle = 10 impulses + ~45" hold; b. 10 impulses + ~45" hold; c. 10 impulses + ~45" hold; d. 10 impulses + ~45" hold; e. 10 impulses + ~45" hold).

(3) Centrifuge at about 30-40000 x g for 15-20 min. E.g.: use rotor Beckman JA 25.50 at 21000 rpm, for 15 min.

(4) Store the centrifugation pellets at -20°C, and load the supernatants on the chromatography columns, as follows

(5) Equilibrate the Poly-Prep (Bio-Rad) columns with 0,5 ml (≅1 ml suspension) of Glutathione-Sepharose 4B resin, wash with 2 ml (1 + 1) H₂O, and then with 10 ml (2 + 4 + 4) PBS, pH 7,4.

(6) Load the supernatants on the columns and discard the flow through.

(7) Wash the columns with 10 ml (2 + 4 + 4) PBS, pH 7.4.

(8) Elute the proteins bound to the columns with 4.5 ml of 50 mM TRIS buffer, 10 mM reduced glutathione, pH 8.0, adding 1.5 ml + 1.5 ml + 1.5 ml and collecting the respective 3 fractions of ~1.5 ml each.

(9) Measure the protein concentration of the first two fractions with the Bradford method, analyse a 10 µg aliquot of proteins from each sample by SDS-PAGE. (N.B.: if the sample is too diluted load 21 µl (+ 7 µl loading buffer)).

(10) Store the collected fractions at +4°C while waiting for the results of the SDS-PAGE analysis.

(11) For each protein destined to the immunisation prepare 4-5 aliquots of 100 µg each in 0.5 ml of 40% glycerol. The dilution buffer is 50 mM TRIS.HCl, 2 mM DTT, pH 8.0. Store the aliquots at -20°C until immunisation.

4. Murine Model of Protection from GAS Infection

(a) *Immunization protocol*

Groups of 10 CD1 female mice aged between 6 and 7 weeks are immunized with two or more GAS antigens of the invention, (20 µg of each recombinant GAS antigen), suspended in 100 µl of suitable solution. Each group receives 3 doses at days 0, 21 and 45. Immunization is performed through intraperitoneal injection of the protein with an equal volume of Complete Freund's Adjuvant (CFA) for the first dose and Incomplete Freund's Adjuvant (IFA) for the following two doses. In each immunization scheme negative and positive control groups are used.

For the negative control group, mice are immunized with *E. coli* proteins eluted from the purification columns following processing of total bacterial extract from a *E. coli* strain containing either the pET21b or the pGEX-NNH vector (thus expressing GST only) without any cloned GAS ORF (groups can be indicated as HisStop or GSTStop respectively).

For the positive control groups, mice are immunized with purified GAS M cloned from either GAS SF370 or GAS DSM 2071 strains (groups indicated as 192SF and 192DSM respectively).

Pooled sera from each group is collected before the first immunization and two weeks after the last one. Mice are infected with GAS about a week after.

Immunized mice are infected using a GAS strain different from that used for the cloning of the selected proteins. For example, the GAS strain can be DSM 2071 M23 type, obtainable from the German Collection of Microorganisms and Cell Cultures (DSMZ).

For infection experiments, DSM 2071 is grown at 37° C in THY broth until OD₆₀₀ 0.4. Bacteria are pelleted by centrifugation, washed once with PBS, suspended and diluted with PBS to obtain the appropriate concentration of bacteria/ml and administered to mice by intraperitoneal injection. Between 50 and 100 bacteria are given to each mouse, as determined by plating aliquots of the bacterial suspension on 5 THY plates. Animals are observed daily and checked for survival.

5. Analysis of Immune Sera

(a) *Preparation of GAS total protein extracts*

Total protein extracts are prepared by incubating a bacterial culture grown to OD₆₀₀ 0.4-0.5 in Tris 50mM pH 6.8/mutanolysin (20 units/ml) for 2 hr at 37° C, followed by incubation for ten minutes on ice in 0.24 N NaOH and 0.96% β-mercaptoethanol. The extracted proteins are precipitated by addition of trichloroacetic acid, washed with ice-cold acetone and suspended in protein loading buffer.

(b) *Western blot analysis*

Aliquots of total protein extract mixed with SDS loading buffer (1x: 60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerin, 0.1% Bromophenol Blue, 100 mM DTT) and boiled 5 minutes at 95° C, were loaded on a 12.5% SDS-PAGE precast gel (Biorad). The gel is run using a SDS-PAGE running buffer containing 250 mM TRIS, 2.5 mM Glycine and 0.1 %SDS. The gel is electroblotted onto nitrocellulose membrane at 200 mA for 60 minutes. The membrane is blocked for 60 minutes with PBS/0.05 % Tween-20 (Sigma), 10% skimmed milk powder and incubated O/N at 4° C with PBS/0.05 % Tween 20, 1% skimmed milk powder, with the appropriate dilution of the sera. After washing twice with PBS/0.05 % Tween, the membrane is incubated for 2 hours with peroxidase-conjugated secondary anti-mouse antibody (Amersham) diluted 1:4000. The nitrocellulose is washed three times for 10 minutes with PBS/0.05 % Tween and once with PBS and thereafter developed by Opti-4CN Substrate Kit (Biorad).

(c) *Preparation of Paraformaldehyde treated GAS cultures*

A bacterial culture grown to OD₆₀₀ 0.4-0.5 is washed once with PBS and concentrated four times in PBS/0.05 % Paraformaldehyde. Following 1 hr incubation at 37° C with shaking, the treated culture is kept overnight at 4° C and complete inactivation of bacteria is then controlled by plating aliquots on THY blood agar plates.

(d) *FACS analysis of Paraformaldehyde treated GAS cultures with mouse immune sera*

About 10⁵ Paraformaldehyde inactivated bacteria are washed with 200 µl of PBS in a 96 wells U bottom plate and centrifuged for 10 min. at 3000g, at 4°C. The supernatant is discarded and the bacteria are suspended in 20 µl of PBS-0.1%BSA. Eighty µl of either pre-immune or immune mouse sera diluted in PBS-0.1%BSA are added to the bacterial suspension to a final dilution of either 1:100, 1:250 or 1:500, and incubated on ice for 30 min. Bacteria are washed once by adding 100 µl of PBS-0.1%BSA, centrifuged for 10 min. at 3000g, 4°C, suspended in 200 µl of PBS-0.1%BSA, centrifuged again and suspended in 10 µl of Goat Anti-Mouse IgG, F(ab')₂ fragment specific-R-Phycoerythrin-conjugated (Jackson Immunoresearch

Laboratories Inc., cat.N°115-116-072) in PBS-0.1%BSA to a final dilution of 1:100, and incubated on ice for 30 min. in the dark. Bacteria are washed once by adding 180 µl of PBS-0.1%BSA and centrifuged for 10 min. at 3000g, 4°C. The supernatant is discarded and the bacteria were suspended in 200 µl of PBS.

Bacterial suspension is passed through a cytometric chamber of a FACS Calibur (Becton Dickinson,

5 Mountain View, CA USA) and 10.000 events are acquired. Data are analysed using Cell Quest Software (Becton Dickinson, Mountain View, CA USA) by drawing a morphological dot plot (using forward and side scatter parameters) on bacterial signals. An histogram plot is then created on FL2 intensity of fluorescence log scale recalling the morphological region of bacteria.

EXAMPLE 2: Comparison of virulence of wild type GAS strain (including GAS 40) and GAS 40 deletion mutant.

The following example provides a comparison between the virulence of a wild type GAS strain and a GAS 40 deletion mutant. Mutant GAS strains where a majority of the GAS 40 sequence is removed were prepared by standard methods. Immunization groups of ten mice per group were injected with either the wild type or mutant GAS strains. As shown below, injection of a range of concentrations of the wild type isolate resulted in mouse fatalities, while injection with the GAS Δ40 mutant did not.

GAS strain	concentration	number of fatalities
wild type	2×10^5	10
wild type	2×10^6	9
wild type	2×10^7	5
GAS Δ40	2×10^2	0
GAS Δ40	2×10^3	0
GAS Δ40	2×10^4	0
GAS Δ40	2×10^5	0
GAS Δ40	2×10^6	0
GAS Δ40	2×10^7	0

EXAMPLE 3: Bacterial Opsonophagocytosis assay of GAS 40 constructs

The following example demonstrates the surface exposure of GAS 40 by use in a bacterial opsonophagocytosis assay. The following GAS constructs, each of which is described in detail above, were used in the assay: 40a-CH, 40a-RR-NH, 40a-RR, GST-40, 40a, 40a and 40a-NH. (The two references to “40a” in Figure 7 refer to sera prepared on different days.

The assay was performed as follows.

1. Preparation of bacterial inoculum. GAS bacteria are grown in THY medium until they reach the middle exponential phase (OD_{600} 0.4) at 37°C. Bacteria are washed twice in chilled saline solution and are suspended in HBSS medium with the volume being adjusted for each strain depending on the amount of bacteria which will be used. Bacterial cells are kept in ice until use.

2. Preparation of PMN. PMN are prepared from buffy coats of heparinized blood from healthy volunteers. The buffy coat is incubated for 30 minutes in a solution containing dextran, NaCl and

Heparin (rate 1:1). After incubation the supernatant, rich of leukocytes, is removed, transferred in a clean tube and centrifuged at 700xg for 20 minutes. A short wash in water is performed to break red blood cells and then a solution of NaCl is added to restore the appropriate salt concentration. After this step cells are centrifuged, washed and suspended in MEM at a suitable concentration.

- 5 3. Opsonophagocytosis assay. GAS strains (prepared as described) are incubated with heat inactivated immune mice serum derived from immunization with the indicated GAS antigen (or preimmune for the control), human PMN and baby rabbit complement. 1 hour of incubation at 37°C. Samples taken immediately before and after the incubation are plated on THY blood agar plates. Phagocytosis is evaluated comparing the difference in the number of colonies at the two times for the
10 preimmune and the immune serum. Data are reported as logarithm number of grown colonies at t=0 - logarithm number of grown colonies at t=60

The results of the assay are shown in Figure 7. The Y axis reports the difference between the logarithm of colony counts at time 0 and the logarithm of the colony counts after 60 seconds: $\log(\text{CFU @ T0}) - \log(\text{CFU @ T60})$. If there has been growth (*i.e.*, the bacteria are not actively killed), negative numbers (negative bars) result. If bacteria are killed, positive numbers (positive histogram bars) result. As shown in
15 Figure 7, positive histogram bars are reported for each of the GAS constructs. The last four yellow bars in Figure 7 represent controls: **B**= bacteria alone, **B PMN**= bacteria + polymorphonucleates, **B C**= Bacteria + complement, **P PMN C**= bacteria + polymorphonucleates + complement (no serum).

EXAMPLE 4: GAS 40 immunization challenge experiments in murine mouse model of protection

20 A sample of the percent survival results from numerous murine mouse model experiments using the GAS 40 antigen are listed below. Annotations indicate where construct used to express the recombinant GAS 40 antigen was modified to facilitate expression.

GAS antigen	% Survival in Mouse Challenge Model
40a	55
40a-RR	70
40a-RR-NH	60

25 It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

CLAIMS:

1. An immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to ten GAS antigens, wherein said combination includes GAS 40.
2. The composition of claim 1, wherein said combination of GAS antigens further includes one or
5 more GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.
3. The composition of claim 1, wherein said combination of GAS antigens includes GAS 117.
4. The composition of claim 1, wherein said GAS 40 antigen comprises an amino acid sequence comprising a first coiled-coil region and a second coiled-coil region.
- 10 5. The composition of claim 1, wherein the GAS 40 antigen comprises an amino acid sequence comprising a first coiled-coil region.
6. The composition of claim 5, wherein said first coiled-coil region comprises an amino acid sequence comprising SEQ ID NO: 12.
7. The composition of claim 4, wherein the GAS 40 antigen comprises an amino acid sequence
15 comprising a second coiled-coil region.
8. The composition of claim 7, wherein said second coiled-coil region includes a leucine zipper region.
9. The composition of claim 7, wherein the second coiled-coil region comprises an amino acid sequence comprising SEQ ID NO: 13.
- 20 10. An immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of a first antigen group, said first antigen group consisting of GAS 117, GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS
25 384, GAS 202, and GAS 057.
11. The immunogenic composition of claim 10, wherein said combination of GAS antigens is selected from the group consisting of GAS 39, GAS 40, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.
12. The immunogenic composition of claim 10, wherein said combination of GAS antigens
30 includes GAS 40 and GAS 117.

13. The immunogenic composition of claim 10, wherein said combination includes GAS 40.
14. The immunogenic composition of claim 10, wherein said GAS 40 is selected from the amino acid sequence comprising (a) a first coiled-coil region, (b) a second coiled-coil region or (c) a first coiled-coil region and a second coiled-coil region.
- 5 15. A fusion construct comprising a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of a first antigen group, said first antigen group consisting of GAS 117, GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS 384, GAS
10 202, and GAS 057.
16. The fusion construct of claim 15, wherein said combination includes GAS 40.
17. The fusion construct of claim 15, wherein said combination includes GAS 40 and GAS 117.
18. A composition comprising a combination of two or more antibodies selected from the group consisting of antibodies specific to antibodies comprising an antibody specific to GAS 40, GAS 117,
15 GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS 384, GAS 202, and GAS 057.
19. The composition of claim 18, wherein said combination includes an antibody specific to GAS 40.
- 20 20. The composition of claim 19, wherein said GAS 40 specific antibody is specific to the first or the second coiled-coil region of GAS 40.
21. A method for the therapeutic or prophylactic treatment of Streptococcus pyogenes infection in an animal susceptible to streptococcal infection comprising administering to said animal a therapeutic or prophylactic amount of any one of the immunogenic compositions of claims 1 to 20.
- 25 22. A method of manufacturing any one of the immunogenic compositions of claims 1 to 20.
23. A kit comprising a first component comprising any one of the immunogenic compositions of claims 1 to 20.
24. A composition comprising a GAS 40 antigen, wherein said antigen comprises an amino acid sequence comprising a first coiled-coil region or a second coiled-coil region.
- 30 25. The composition of claim 24, wherein said GAS 40 antigen comprises a first coiled-coil region comprising SEQ ID NO: 12.

26. The composition of claim 24, wherein said GAS 40 antigen comprises a second coiled-coil region comprising SEQ ID NO: 13.

27. An antibody specific to any one of the compositions of claims 24 to 26.

FIGURE 1: Annotation of GAS 40

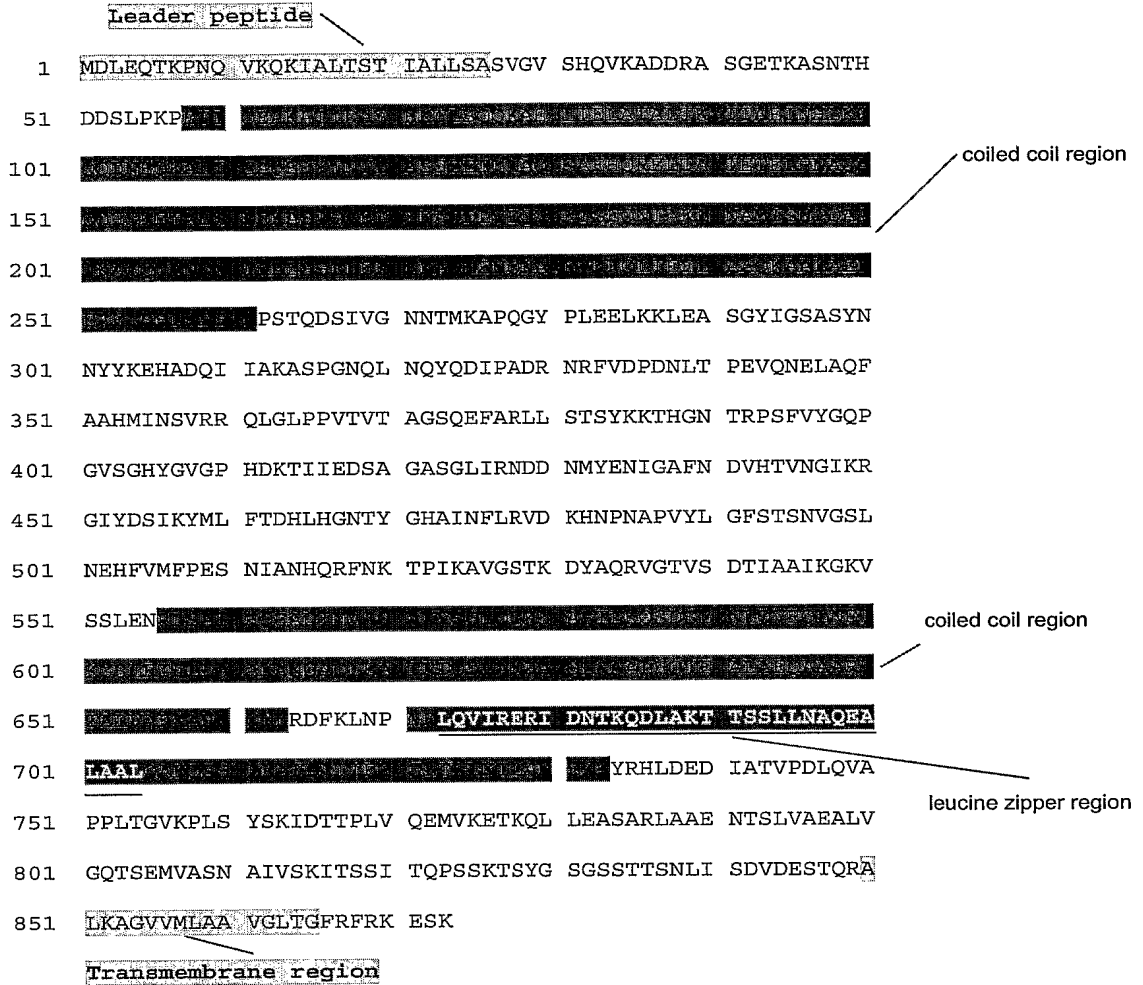


FIGURE 2 : Schematic of GAS40: putative surface exclusion protein prgA (873aa)

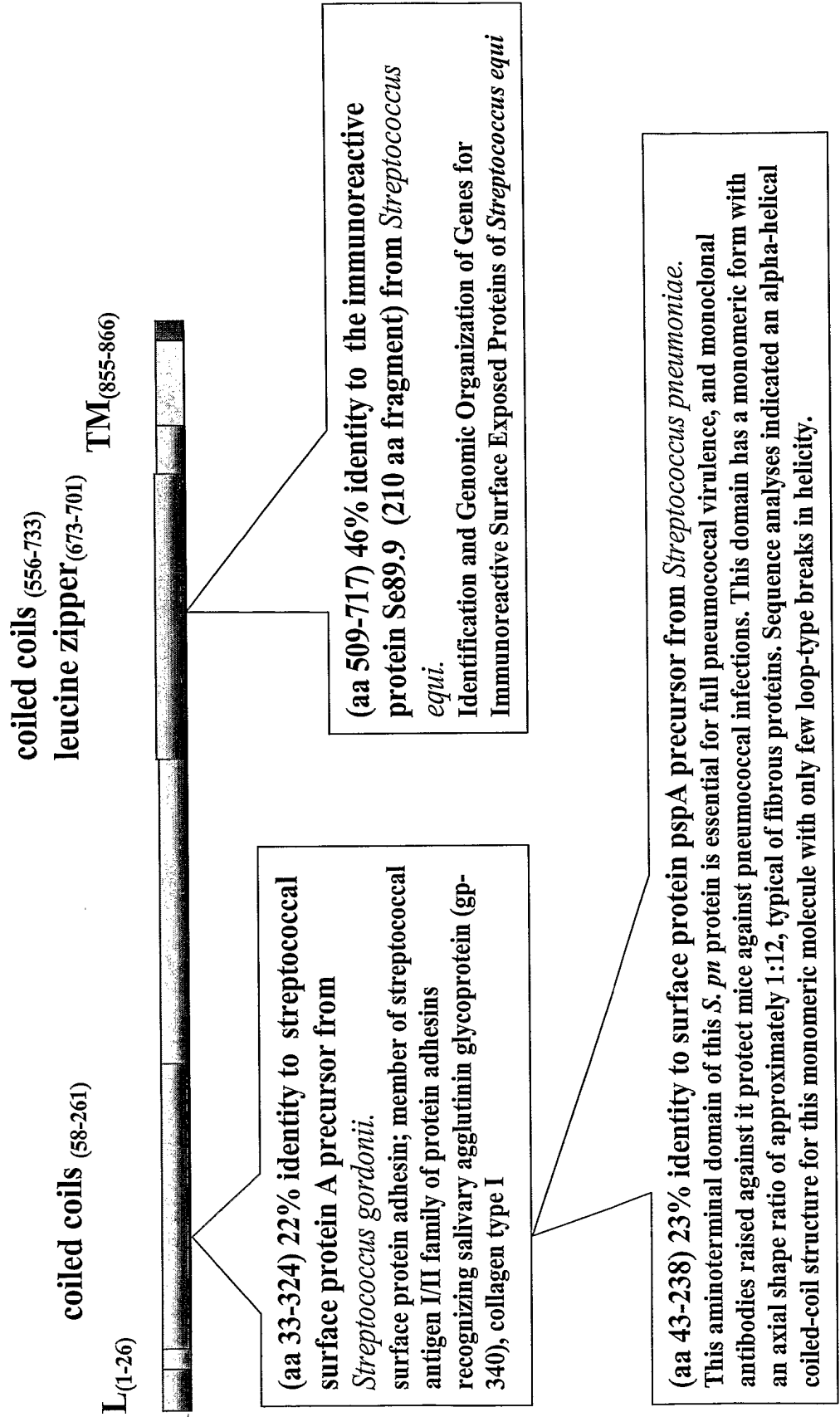


FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(a) BLAST alignment of amino acid sequence of GAS 40 including the first coiled-coil region with SpA precursor of *Streptococcus gordonii*

```
>gi|25990270|gb|AAC44101.3| streptococcal surface protein A precursor
[Streptococcus gordonii]
    Length = 1575
>ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]
    Length = 873
```

```
Score = 63.2 bits (152), Expect = 5e-11
Identities = 65/293 (22%), Positives = 124/293 (42%), Gaps = 13/293
(4%)
```

```
Query: 112 QDQTSKGTATTAAENAQKQAEIKSDYAKQA----EEIKKTTEAYKKEVEAHQAETDKIN
167
```

```
          Q + D+ + T A N + K + ++A + ++KT K E+ K
Sbjct: 33 QVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVEKTL SQQKAE LTELATALTKTT
92
```

```
Query: 168 AENKAAEDKYQEDLKAHQAEVEKINTANATAKAEYEAKLAQYQKDLAAVQKANEDSQLDY
227
```

```
          AE +++ + KA + E A+++ A+ A++Q++L A + ++Q D
Sbjct: 93 AEINHLKEQQDNEQKAL TSAQE IYTN TLASSEETLLAQGAEHQRELTATETELHNAQADQ
152
```

```
Query: 228 QNKLSAYQAE LARVQKANAEAKEAYE--KAVKENTAKNAALQAENEAIKQRNETAKANYD
285
```

```
          +K +A + A + A++ E K ++N AK A+ + +AI + +TA N
Sbjct: 153 HSKETAL SEQKASISAETTRAQDLVEQVKTSEQNI AKLNAMISNPDAITKAAQTANDNTK
212
```

```
Query: 286 AAMKQYEADLAAIKKAKEDNDADYQAKLAA YQAE LARVQKANADAKAAYEKAVEENTAKN
345
```

```
          A + E A ++ K +LAA +A LA + + K++ + N
Sbjct: 213 ALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAEL SRLKSSAPSTQDSIVGNN
272
```

```
Query: 346 TAIQAEN---EAIKQRNAA---AKATYEAALKQYEADLAAAKKANEDSDADYQ 392
```

```
          T + E +K+ A+ A+Y K++ AD AK + + YQ
Sbjct: 273 TMKAPQGYPLEELKKLEASGYIGSASYN NYKEH-ADQIIAKASPGNQLNQYQ 324
```

FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(b) BLAST alignment of amino acid sequence of GAS 40 including the first coiled-coil region with SpB precursor of *Streptococcus gordonii*

```
>gi|25055226|gb|AAC44102.3| streptococcal surface protein B precursor
[Streptococcus gordonii]
      Length = 1499
>ref|NP_268623.1| putative surface exclusion protein [Streptococcus
pyogenes]
      Length = 873
```

```
Score = 54.3 bits (129), Expect = 2e-08
Identities = 53/226 (23%), Positives = 98/226 (43%), Gaps = 13/226
(5%)
```

```
Query: 111 QDQTSDKGTATTAENAQKQAEIKSDYAKQA----EEIKKTTEAYKKEVEAHQAETDKIN
166
```

```
      Q + D+ + T A N + K + ++A + ++KT K E+ K
Sbjct: 33 QVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVEKTLSQKQAEKTELATALTKIT
92
```

```
Query: 167 AENKAAEDKYQEDLKAHQAEVEKINTANATAKAEYEAKLAQYQKDLAAVQKANEDSQLDY
226
```

```
      AE +++ + KA + E A+++ A+ A++Q++L A + ++Q D
Sbjct: 93 AEINHLKEQQDNEQKAL TSAQEIYTNTLASSEETLLAQGAEHQRELTATETELHNAQADQ
152
```

```
Query: 227 QNKLSAYQAELARV--QXXXXXXXXXXXXXXXXXNTAKNAALQAENEAIKQRNETAKANYD
284
```

```
      +K +A + A + + N AK A+ + +AI + +TA N
Sbjct: 153 HSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDNTK
212
```

```
Query: 285 AAMKQYE---ADL----AAIKKAKEDNDADYQAKLAAYQAELARVQ 323
```

```
      A + E ADL A +KK + A +A LA +AEL+R++
Sbjct: 213 ALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRLK 258
```

FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(c) BLAST alignment of amino acid sequence of GAS 40 including the first coiled-coil region with Surface Protein PspA precursor of *Streptococcus pneumoniae*

```
>gi|282335|pir|A41971 surface protein pspA precursor - Streptococcus pneumoniae
>ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]
```

Length = 873

Score = 48.1 bits (113), Expect = 6e-07
 Identities = 46/200 (23%), Positives = 89/200 (44%), Gaps = 23/200 (11%)

```
Query: 139 KTKFNTVRAMVVPEPEQLAETK-----KKSEEAKQKAPELTKKLEEKAKLEE-AEKK
190
```

```
      +TK +      +P+PE + E K      K + K + EL L + A++ E++
Sbjct: 43 ETKASNTHDDSLPKPETIQEAKATIDAVEKTLSSQQAELTELATALTKTTAEINHLKEQQ
102
```

```
Query: 191 ATEAKQKVDAEEVAPQAKIAELENQVHRLEQELKEIDSESESEDYAKEGFRAPLQSKLDAK
250
```

```
      E K A+E+      + E + + + +E+ +E+E + + + ++ L +
Sbjct: 103 DNEQKAL TSAQEIIYTN TLASSEETLLA QGAHQRELTATETELHNAQADQHSKETALSEQ
162
```

```
Query: 251 KAKLS----KLEELSDKIDELDAEIAKLEDQL-----KAAEENNNVEDYFKEGLEKTI
299
```

```
      KA +S      + ++L +++ + IAKL +      KAA+ N+      LEK
Sbjct: 163 KASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDNTKALSSELEKA-
221
```

```
Query: 300 AAKKAELEKTEADLKKAVNE 319
```

```
      KA+LE +A +KK + E
Sbjct: 222 ---KADLENQKAKVKKQLTE 238
```

FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(d) BLAST alignment of amino acid sequence of GAS 40 including the second coiled-coil region with SpB precursor of *Streptococcus gordonii*

>gi|23380384|gb|AAN18299.1| immunoreactive protein Se89.9 (fragment) [Streptococcus equi]

Length = 210

>ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]

Length = 873

Score = 173 bits (438), Expect = 4e-45
Identities = 98/209 (46%), Positives = 144/209 (68%)

Query: 1 ESDIVDATRFSTTEIPKSGQVIDRSASIQALTNDIASIKGKIASLESRLADPSSEAEVTA
60

ES+I + RF+ T I G D + + +++ IA+IKGK++SLE+RL+ EA++ A
Sbjct: 509 ESNIANHQRFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGVSSLENRLSAIHQEADIMA
568

Query: 61 AQAKISQLQHQLAAQAKSHKLDQQVEQLANTKDSLRTQLLAAKEEQAQLKANLKDALAL
120

AQAK+SQLQ +L + +S L+ QV QL +TK SLRT+LLAAK +QAQL+A D++LA
Sbjct: 569 AQAKVSQLQGKLASTLKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAK
628

Query: 121 LASSKATLHKLEAAMEEAKARVAGLASQKAQLEDLLAFEKNPNRIELAQEKVAAAKKALA
180

LAS KA LH+ EA E+A ARV L ++KA L+ L F+ NPNR+++ +E++ K+ LA
Sbjct: 629 LASLKAALHQTEALAEQAAARVTALVAKKAHLQYLRFKLNPNRLQVIRERIDNTKQDLA
688

Query: 181 DTEDKLLAAQASLSDLQAQRARLQLSIAT 209

T LL AQ +L+ LQA+++ L+ +IAT
Sbjct: 689 KTTSSLLNAQEALAAALQAKQSSLEATIAT 717

Figure 4: Secondary Structure Prediction of GAS 40

Figure 4(a) Secondary Structure prediction alignment with GAS 40 amino acid sequence

```

      10      20      30      40      50      60      70
      |      |      |      |      |      |      |
MDLEQTKPNQVKQKIALTSTIALLSASVGVSHQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAV
CCCCCCCCchhhHHhhhhHHHHhhccccEEEEeCCCCCCCCcCCCCCCCCcCHHHHHHHHHHH
EKTLSQQKAELTELATALTKTTAEINHLKEQQDNEQKALTSAQBIYTNTLASSEETLLAQGAHQRELT
HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHhhccccHHHHHHHHHHHHHH
TETELHNAQADQHSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDN
HHHHHHHHHHccccchHHHHhhhhccccchhhHHHHHHHHHHHHHHHHHHHHhhccccCHHHHHHHHHhhc
TKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRLKSSAPSTQDSIVGNNTMKAPQGY
cHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHhhccccCCCCCCCC
PLEELKLEASGYIGSASYNYYKEHADQIIAKASPGNQLNQYQDIPADRNRFDVDPDNLTPEVQNELAQF
CHHHHHHHhhcCccccccccchHHHHHHHHHHHHhhCChhhhhhhccccccccCCCCCCCCChHHHHHHHH
AAHMINSVRRQLGLPPVTVTAGSQEFARLLSTSYKKTGHGTRPSFVYQPGVSGHYGVGPHDKTIIEDSA
HHHHHHHHHHhhCCCCccecCHHHHHHHHHhhccccCCCCcEEBcCCcceecceCcCCcEEEEcC
GASGLIRNDDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYGHAINFLRVDKHNPNAPVYL
CCCceecCCchHHhhhhccccccccCccccHHHHHHHHhhccccCccchHHheeeecCCCCCCEEE
GFSTSNVGSLSNEHFVMPESNIANHQRFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGVSSLENRLSAI
EEEcCccCccccccccccccchHHhhCCCCCCCCcCHHHHHHHchhHHHHHHhhcCccHHHHHHHH
HQEADIMAAQAKVSQLQGLASTLQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLA
HHHHHHHHHHHHHHHHHHHHHHHHhhccccChhhhhhhhhcCcCHHHHHHHHHHHHHHHHHHHHHHH
SLKAALHQTEALAEQAAARVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTTSSLLNAQEA
HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHCCCCCHHHHHHHHHHHHHHHHH
LAALQAKQSSLEATIATTEHQTLTKLANEKEYRHLDEDIATVPDLQVAPPLTGVKPLSYSKIDTTPLV
HHHHHHhhcCceecccccchHHHHHHHHHHHHhhhhhhHHHHhhccccCCCCcCCCCCcCCCCceecCCHHH
QEMVKETKQLLEASARLAAENTSLVAEALVQGQTSSEMVASNAIVSKITSSITQPSSKTSYSGSGSSTTSLNI
HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHhhccccchHHHHhhchhhhhccccEEEcCCCCccccccccCcccCccc
SDVDESTQRALKAGVVMLAAVGLTGFRRFRKESK
cCCchHHHHHHhhccccEEccccccccccccCC

```

Sequence length : 873

PHD :

Alpha helix	(Hh)	:	525	is	60.14%
3 ₁₀ helix	(Gg)	:	0	is	0.00%
Pi helix	(Ii)	:	0	is	0.00%
Beta bridge	(Bb)	:	0	is	0.00%
Extended strand	(Ee)	:	63	is	7.22%
Beta turn	(Tt)	:	0	is	0.00%
Bend region	(Ss)	:	0	is	0.00%
Random coil	(Cc)	:	285	is	32.65%
Ambiguous states (?)		:	0	is	0.00%

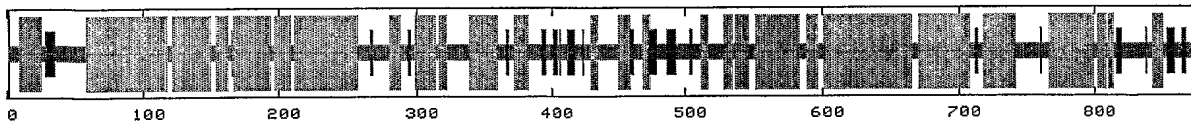
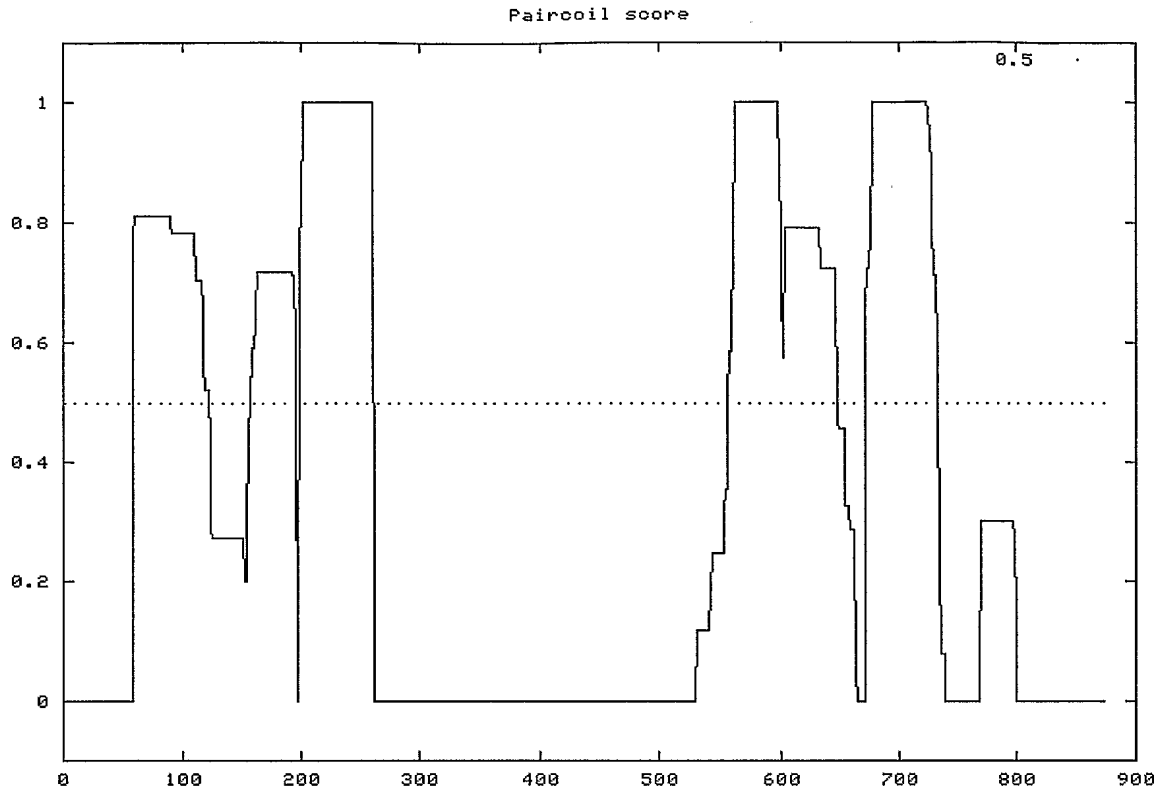


Figure 4(b): Secondary Structure prediction based on PairCoil Score



Coiled coils found:

```

positions 58- 121   initial register 'f'   probability 0.811
positions 156- 196   initial register 'e'   probability 0.720
positions 198- 246   initial register 'e'   probability 1.000
positions 247- 261   initial register 'a'   probability 1.000

positions 556- 646   initial register 'e'   probability 1.000
positions 671- 733   initial register 'f'   probability 1.000

```

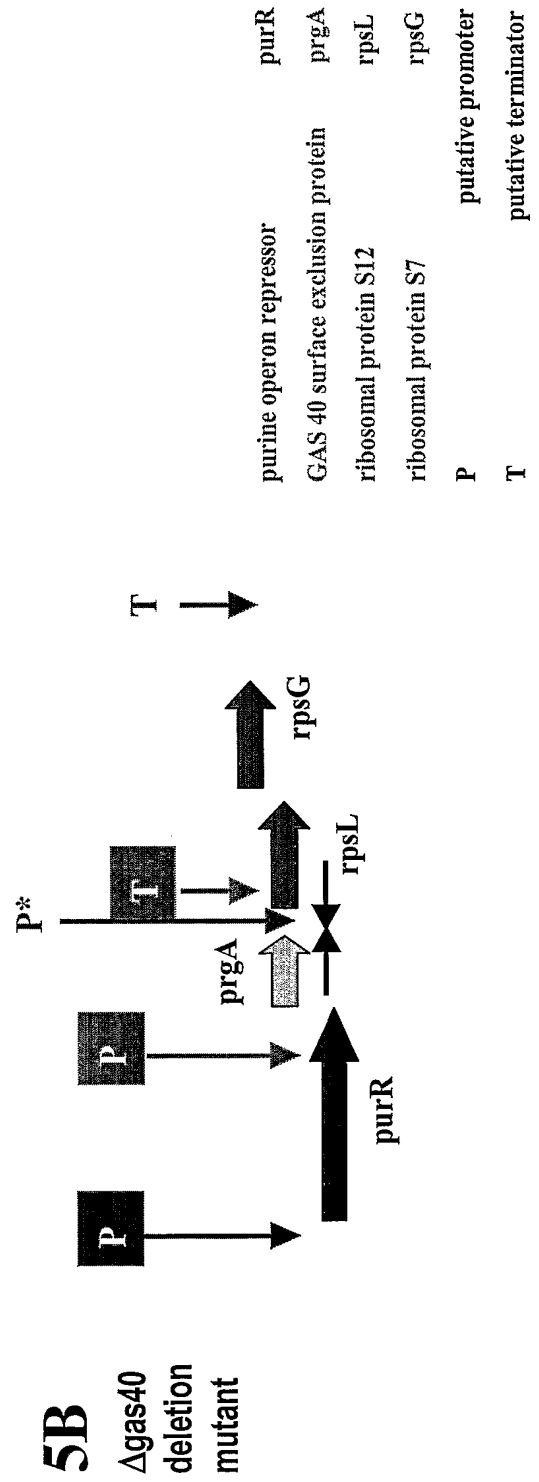
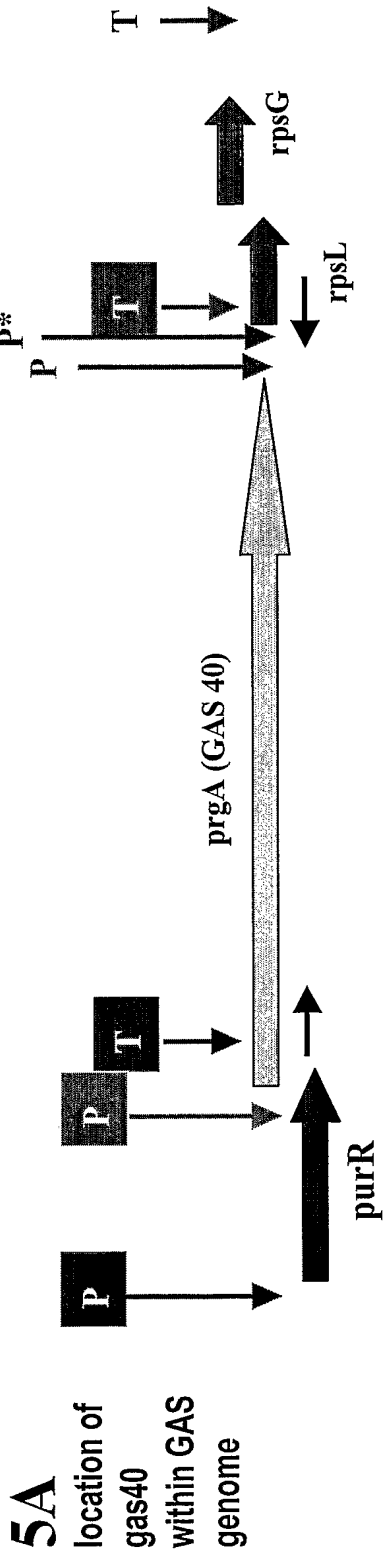
Figure 4(c): Secondary Structure prediction of Leucine Zipper within coiled coil.

```

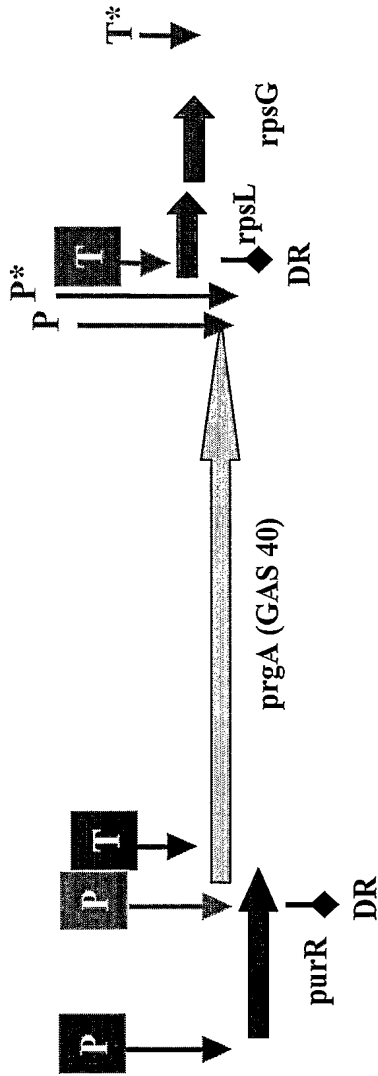
           673                               701
QYLRDFKLNPNRLQVIRERIDNTKQDLAKTTSSLLNAQEALALQAKQSSLEATIATTEH
          CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
          L-----I-----L-----L-----L
          OOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOO

```

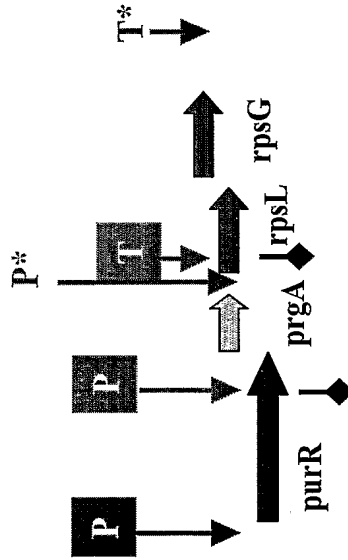
Figure 5



5C
 direct repeats
 surrounding GAS 40
 within wild type genome



5D
 location of direct
 repeats within Δ gas40



5E
 corresponding
 genomic region
 within GBS

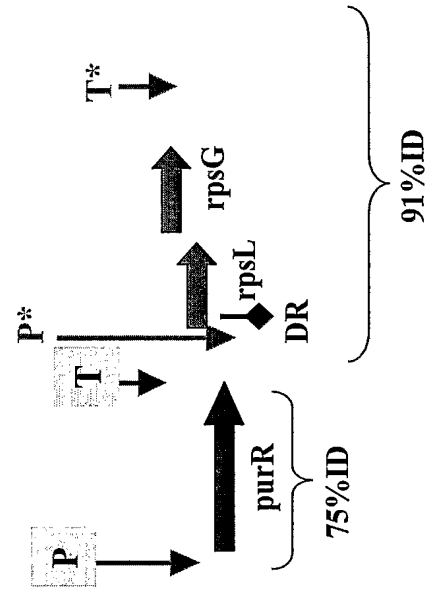


FIGURE 6: FACS Comparison of GAS 40 in wild type GAS and GAS 40 deletion mutant

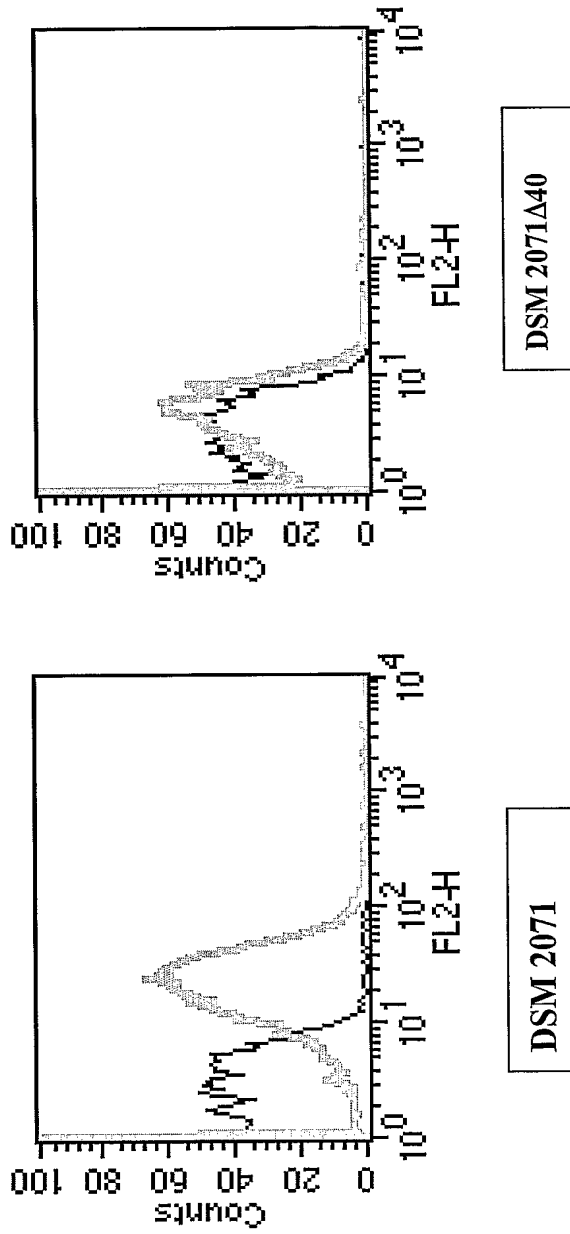
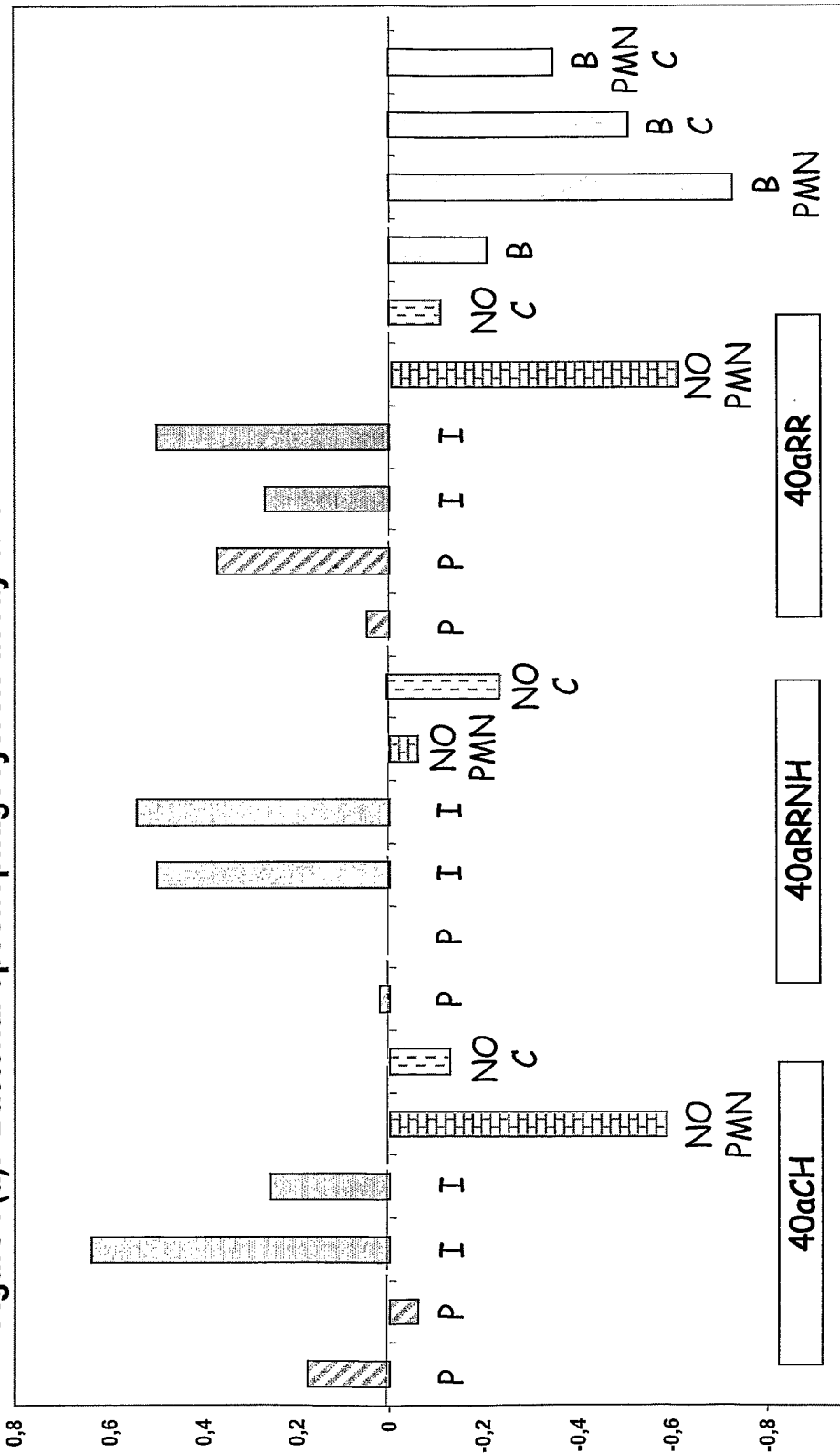


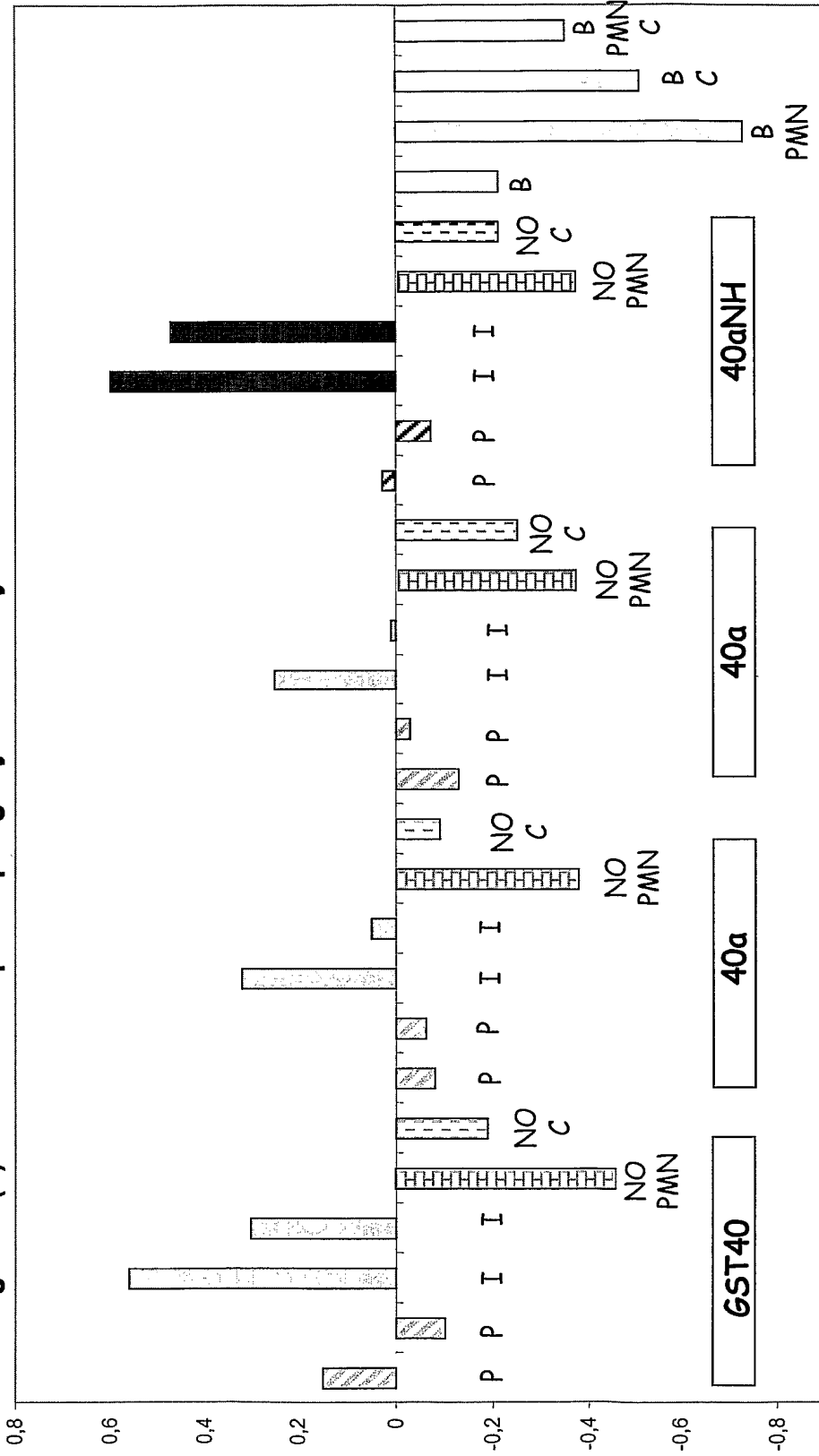
Figure 7(a): Bacterial opsonophagocytosis assay of GAS 40 constructs



B - bacteria
 P - pre-immune serum
 I - immune serum
 No PMN - without polymorphonucleates
 No C - without complement

Histogram bars represent the difference between logarithm at T 0 (initial time) and T60 (bacterial CFU counted after 60 minutes of incubation)

Figure 7(b): Bacterial opsonophagocytosis assay of GAS 40 constructs



B - bacteria
 P - preimmune serum
 I - immune serum
 No PMN - without polymorphonucleates
 No C - without complement

Histogram bars represent the difference between logarithm at T 0 (initial time) and T60 (bacterial CFU counted after 60 minutes of incubation)

Figure 8: Immunization in Murine Mouse Model

GAS antigen	Survival/Tested mice			Protection %	pValue Chi-square	Protein Purity %
	alive	dead	tested			
gst 40	67	63	130	51	0.00012	
253	14	36	50	28	0.006	15
253-urea	2	8	10	20		25
253-gst	2	8	10	20		30
39	9	31	40	22.5	0.09	20
39a	13	37	50	26	0.016	10
39a	10	30	40	25	0.039	
39a	12	28	40	30	0.0046	
urea 366	21	78	99	21.2	0.046	65
117	19	51	70	27	0.0036	15
117-urea	1	9	10	10		80
117-urea-2M	7	23	30	23.3	0.1	80
117-urea-2M (prep 117)	8	32	40	20	0.2	
urea 504	9	31	40	22.5	0.09	50
504	14	26	40	35	0.0003	40
504	7	33	40	17.5	0.4	80
urea 389	7	23	30	23	0.1	30
533	14	56	70	20	0.12	50
new 533	4	16	20	20	0.34	30
gst 57	12	48	60	20	0.14	60
57a	0	20	20	0		50
294	17	73	90	18.8	0.14	80
130	15	65	80	18.7	0.17	40
130	7	23	30	23.3	0.1	40
84	8	32	40	20	0.2	70
urea 159	7	33	40	17.5	0.4	5
159a	2	8	10	20		65
527	10	40	50	20	0.17	50
527	3	17	20	15		80
217	7	33	40	17.5	0.4	50
511	13	67	80	16.2	0.41	80
277	8	42	50	16	0.52	5
277a	2	28	30	6.6		50
gst 202	3	17	20	10	0.75	5
202a	5	25	30	16.6	0.53	5
45	5	25	30	16.6	0.53	80
urea 309	5	25	30	20	0.53	8
290	6	34	40	15	0.67	50
529	6	34	40	15	0.67	5
gst 58	10	60	70	14.2	0.71	30
384	7	43	50	14	0.78	80
384RR	1	19	20	5		80
urea 509	7	53	60	11.6	0.84	50
509-NH	2	8	10			75
509-CH	0	10	10			75
193	7	53	60	11.6	0.84	65
urea 372	4	25	29	13.7	0.85	20
gst 42	4	26	30	13.3	0.9	50
95	5	35	40	12.5	1	55
urea 236	5	35	40	12.5	1	80
new 236	2	8	10	20		70
137	5	35	40	12.5	1	75
His-Stop	29	201	230	12:06		

SEQUENCE LISTING

SEQ ID NO: 1 amino acid sequence comprising GAS 40

MDLEQTKPNQVKQKIALTSTIALLSASVGVSHQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVE
KTLSSQKAELELATALTKTTAEINHLKEQQDNEQKALTSAQEIYTNNTLASSEETLLAQGAEHQRELTATE
TELHNAQADQHSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDNTKA
LSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRLKSSAPSTQDSIVGNNTMKAPQGYPLEE
LKKLEASGYIGSASYNMYKHEADQIIAKASPGNQLNQYQDIPADRNRFDVDPDNLTPVQNELAQFAAAMI
NSVRRQLGLPPVTVTAGSQEFARLLSTSYKKTHGNTRPSFVYQPGVSGHYGVGPHDKTIIEDSAGASGLI
RNDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYGHAINFLRVDKHNPNA PVYLGFSSTSNV
GSLNEHFVMPFESNIANHQRFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKQKVSLENRLSAIHQEADIMA
AQAKVSQLOGKLASTLKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLASLKAALHQT
EALAEQAAARVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTTSLLNAQEALAAALQAKQSS
LEATIATTEHQLTLLKTLANEKEYRHLDEDIATVPDLQVAPPLTGVKPLSYSKIDTTPLVQEMVKETKQLL
EASARLAAENTSLVAEALVGQTSSEMVASNAIVSKITSSITQPSKTSYSGSGSSTTSNLI SDVDESTQRALK
AGVVMLAAVGLTGFRFRKESK

SEQ ID NO: 2 polynucleotide sequence encoding for GAS 40

ATGGACTTAGAACAAACGAAGCCAAACCAAGTTAAGCAGAAAATTGCTTTAACCTCAACAATTGCTTTTATT
GAGTGCCAGTGTAGGCGTATCTCACCAGTCAAAGCAGATGATAGAGCCTCAGGAGAAAACGAAGGCAGTA
ATACTCACGACGATAGTTTACCAAAACCAGAAACAATTCAAGAGGCAAAGGCAACTATTTGATGCAGTTGAA
AAAACCTCTCAGTCAACAAAAGCAGAACTGACAGAGCTTGCTACCGCTCTGACAAAACCTACTGCTGAAAT
CAACCCTTAAAAGAGCAGCAAGATAATGAACAAAAGCTTTAACCTCTGCACAAGAAAATTTACACTAATA
CTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCTACTGAA
ACAGAGCTTCATAATGCTCAAGCAGATCAACATTTCAAAGAGACTGCATTGTCAGAACAAAAGCTAGCAT
TTCAGCAGAAAACCTCGAGCTCAAGATTTAGTGGAAACAAGTCAAACCGTCTGAACAAAATATTGCTAAGC
TCAATGCTATGATTAGCAATCCTGATGCTATCACATAAGCAGCTCAAACGGCTAATGATAATACAAAAGCA
TTAAGCTCAGAATTGGAGAAGGCTAAAAGCTGACTTAGAAAATCAAAGCTAAAAGTTAAAAGCAATTGAC
TGAAGAGTTGGCAGCTCAGAAAAGCTGCTCTAGCAGAAAAGAGGCAGAACTTAGTCGTCTTAAATCCTCAG
CTCCGCTTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCGCAAGGCTATCCTCTTGAAGAA
CTTAAAATTTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACAAAGAGCATGCAGA
TCAAATTTATTGCCAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGATATTTCCAGCAGATCGTAATC
GCTTTGTTGATCCCATAATTTGACACCAGAAGTGCAAAATGAGCTAGCGCAGTTTGCAGCTCACATGATT
AATAGTGTAAGAAGCAATTTAGGCTTACCACCAGTTACTGTTACAGCAGGATCACAAGAAATTTGCAAGATT
ACTTAGTACCAGCTATAAGAAAACCTCATGGTAATACAAGACCATCATTGTCTACGGCAGCCAGGGGTAT
CAGGGCATTATGGTGTGGGCTCATGATAAAAAC TATTATTGAAGACTCTGCCGGAGCGCTCAGGGCTATT
CGAAATGATGATAACATGTACGAGAATATCGGTGCTTTTTAACGATGTGCATACTGTGAATGGTATTTAAACG
TGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAAATACATACGGCCATGCTA
TTAACTTTTTTACGTGTAGATAAACATAACCTTAATGCGCCTGTTTACCTTGGATTTTCAACCAGCAATGTA
GGATCTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACATTGCTAACCATCAACGCTTTAATAAGAC
CCCTATAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGTAGGCACTGTATCTGATACTATTGCGAG
CGATCAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTGTGCGCTATTTCATCAAGAAGCTGATATTTATGGCA
GCCAAGCTAAAGTAAGTCAACTTCAAGGTAATTTAGCAAGCACACTTAAGCAGTCAGACAGCTTAAATCT
CCAAGTGAGACAATTAATGATACTAAAGTTCTTTTGAGAACAGAAATTAAGCAGCTAAAGCAAAAACAAG
CACAACTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTGAAAGCCGCACTGCACCAGACA
GAAGCTTAGCAGAGCAAGCCGAGCCAGAGTGACAGCAC TGGTGGCTAAAAAAGCTCATTTGCAATATCT
AAGGGACTTTAAATTTGAATCCTAACCGCTTCAAGTGATACGTGAGCGCATTGATAATACTAAGCAAGATT
TGGCTAAAACCTACCTCATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCTAAACAAAGCAGT
CTAGAAGCTACTATTGCTACCACAGAACACCAGTTGACTTTGCTTAAAACCTTAGCTAACGAAAAGGAATA
TCGCCACTTAGACGAAGATATAGCTACTGTGCCGTGATTTGCAAGTAGCTCCACCTCTTACGGGCGTAAAAC
CGCTATCATATAGTAAGATAGATACTACTCCGCTTGTTCAGAAATGGTTAAAGAAAACGAAAACAACCTATTA
GAAGCTTAGCAAGATTAGCTGCTGAAAATACAAGCTTTGTAGCAGAAAGCGCTTGTGGCCAAACCTCTGTA
AATGGTAGCAAGTAAATGCCATTTGTGCTTAAAATCACATCTTCGATTACTCAGCCCTCATCTAAGCATCTT
ATGGCTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTGATGAAAGTACTCAAAGACTCTTAAA
GCAGGAGTCGTATGTTGGCAGCTGTGCGCCTCACAGGATTTAGGTTCCGTAAGGAATCTAAGTGA

SEQ ID NO: 3 amino acid sequence comprising an N terminal leader sequence of GAS 40

MDLEQTKPNQVKQKIALTSTIALLSA

SEQUENCE LISTING

SEQ ID NO: 4 polynucleotide sequence encoding an N terminal leader sequence of GAS 40

ATGGACTTAGAACAAACGAAGCCAAACCAAGTTAAGCAGAAAATTGCTTTAACCTCAACCAATTGCTTTATT
GAGTGCC

SEQ ID NO: 5 amino acid sequence comprising a fragment of GAS 40 with N terminal leader sequence removed

SVGVSHQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVEKTLSSQKAELTELATALTKTTAEINH
LKEQQDNEQKALTSAQEIYTNLASSSEETLLAQGAEHQRELATETELHNAQADQHSKETALSEQKASISA
ETTRAQDLVEQVKTSEQNIAKLNAMI SNPDAITKAAQTANDNTKALSSELEKAKADLENQKAKVKKQLTEE
LAAQKAALAEKEAELSRLKSSAPSTQDSIVGNNTMKAPQGYPLEELKKLEASGYIGSASYNYYKEHADQI
IAKASPGNQLNQYQDIPADRNRFPDNLTPREVQNELAQFAAHMINSVRRQLGLP PVTVTAGSQEFARLLS
TSYKKTHTGNTRPSFVYQPGVSGHYGVGPHDKTIIEDSAGASGLIRNDDNMYENIGAFNDVHTVNGIKRGI
YDSIKYMLFTDHLHGNTYGHAINFLRVDKHNPNAPVYLG FSTSNVGSLSNEHFVMPESNINHQRFNKTP
KAVGSTKDYAQRVGTVSDTIAAIKGVSSLENRLSAIHQEADIMAAQAKVSQLQGLASTLTKQSDSLNLQV
RQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLASLKAALHQTEALAEQAAARVTALVAKKAHLQYLDR
FKLNPNRLOVIRERIDNTKQDLAKTTSLLNAQEALAAQAKSSLEATIATTEHQLTLLKTLANEKEYRHR
LDEDIATVDDLQVAPPLTGVKPLSYSKIDTTPLVQEMVKETKQLLEASARLAAENTSLVAEALVQGTSEMV
ASNAIVSKITSSITQPSSKTSYSGSSSTSNLISDVDESTQRALKAGVVMLAAVGLTGFRFRKESK

SEQ ID NO: 6 polynucleotide sequence encoding a fragment of GAS 40 with N terminal leader sequence removed

AGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAAACGAAGGCGAGTAATACTCA
CGACGATAGTTTACCAAAACCAGAAACAATTCAAGAGGCAAAGGCAACTATTGATGCAGTTGAAAAA
CTCTCAGTCAACAAAAAGCAGAACTGACAGAGCTTGCTACCGCTCTGACAAAACTACTGCTGAAATCA
ACCAC TAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAATTTACACTA
ATACTCTGC AAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAG
CTACTGAAACAGAGC TGCATAATTCACAACATTCAAAAAGAGACTGCATTGTGAGAACA
AAAAAGCTAGCATTTCAGCA GAAACTACTCGAGCTCAAGATTTAGTGGAACAAGTCAAACCGT
CTGAAACAAAATATTGCTAAGCTCAATGC TATGATTAGCAATCCTGATGCTATCACTAAAGC
AGCTCAAACCGCTAATGATAATACAAAAGCATTAAGCT CAGAATTGGAGAAGGCTAAAGCT
GACTTAGAAAATCAAAGCTAAAGTTAAAAAGCAATTGACTGAAGAG TTGGCAGCTCAGAAAG
CTGCTTAGCAGAAAAGAGGCGAAGCTTAGTCGTCTTAAATCCTCAGCTCCGTC TACTCAAG
ATAGCATTGTGGGTAATAATACCATGAAAGCACCAGGCTATCCTCTTGAAGAACTTAAAA
AATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACAAAAGCAGATGC
AGATCAAATT ATTTGCCAAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGATATCCAGC
AGATCGTAATCGCTTTGT TGATCCCAGATAATTTGACACCAGAAGTGCAAAATGAGCTAGC
GCAGTTGCGAGCTCACATTAATAGTG TAAGAAGACAATTAGGTCTACCACAGTTACTGTT
ACAGCAGGATCACAAGAAATTTGCAAGATTACTTAGT ACCAGCTATAAGAAA
ACTCATGGTAATACAAGACCATCATTTGTCTACGGACAGCCAGGGGTATCAGGGCA TTAT
GGTGTGGCCCTCATGATAAACTATTATTGAAGACTCTGCCGGAGCGTCAGGGCTCATTCG
AAATG ATGATAACATGTACGAGAATATCGGTGCTTTTAACGATGTGCATACTGTGAATGGT
ATTAAACGTGGTATT TATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAA
ATACATACGGCCATGCTATTAACCT TTTACGTGTAGATAAAACATAACCTAATGCGCCTG
TTTACCTTGGATTTTCAACCAGCAATGTAGGATCTT TGAATGAACACTTTGTAATGTTCC
AGAGTCAACATTTGCTAACCATCAACGCTTTAATAAGACCCCTATA AAAGCCGTTGGAAGT
ACAAAAGATTATGCCCAAAGAGTAGGCATGTATCTGATACTATTGCAAGCATCAA AGG
AAAAGTAAGTCAACTTCAAGGTAATTTAGCAAGCACACTTAAGCAGTCAGACAGCTTAAAT
CTCCAAGTG AGACAATTAATGATACTAAAGGTTCTTTGAGAACAGAATTACTAGCAGCTAA
AGCAAAACAAGCACA ACTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTG
AAAGCCGCACTGCACCAGACAGAAGCCT TAGCAGAGCAAGCCGAGCCAGAGTGACAGCA
CTGGTGGCTAAAAAGCTCATTTGCAATATCTAAGGGAC TTTAAATTTGAATCCTAACCG
CCTTCAAGTGATACGTGAGCGCATTGATAATACTAAGCAAGATTTGGCTAA AACTACCT
CATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCTAAAACAAGCAGTCTAG
AAGCTACTATTGCTACCACAGAACACCAGTTGACTTTGCTTAAAACCTTAGCTAACGAAA
AGGAATATCGCCAC TTAGACGAAGATATAGTACTGTGCCTGATTGCAAGTAGCTCCAC
CTTACGGCGTAAAACCGCTATC ATATAGTAAGATAGATACTACTCCGCTTGTTC
AAGAAATGGTTAAAGAAACGAAACAAC TATTAGAAGCTT CAGCAAGATTAGCTGCTG
AAAAATACAAGTCTTTGTAGCAGAAGCGCTTGTGGCCAAACCTCTGAAATGGTA
GCAAGTAATGCCATTGTGCTAAAAATCACATCTTCGATTACTCAGCCCTCATCTAAGACAT
CTTATGGCTC

SEQUENCE LISTING

AGGATCTTCTACAACGAGCAATCTCATTCTGATGTTGATGAAAGTACTCAAAGAGCTCTTAAAGCAGGAG
TCGTCATGTTGGCAGCTGTCCGCCCTCACAGGATTTAGGTTCCGTAAGGAATCTAAGTGA

SEQ ID NO: 7 amino acid sequence comprising a C terminal transmembrane region of GAS 40
ALKAGVVMLAAVGLTGFRFRKESK

SEQ ID NO: 8 polynucleotide sequence encoding a C terminal transmembrane region of GAS 40
GCTCTTAAAGCAGGAGTCGTCATGTTGGCAGCTGTCCGCCCTCACAGGATTTAGGTTCCGTAAGGAATCTAA
GTGA

SEQ ID NO: 9 amino acid sequence comprising a fragment of GAS 40 with a C terminal
transmembrane sequence removed

MDLEQTKPNQVKQKIALTSTIALLSASVGVSHQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVE
KTLSQQKAELTELATALTKTTAEINHLKEQQDNEQKAL TSAQEIYTNTLASSEETLLAQGAHQREL TATE
TELHNAQADQHSKETALSEQKASISAETTRAQDLVEQVKTSEQNI AKLNAMI SNPDAITKAAQTANDNTKA
LSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRLKSSAPSTQDSIVGNNTMKAPQGYPLEE
LKKLEASGYIGSASYNYYKEHADQIIAKASPGNQLNQYQDIPADRNR FVDPDNLTPEVQNELAQFAAHI
NSVRRQLGLPPVTVTAGSQEFARLLSTSYKKTHGNTRPSFVYVGPVSGHYGVGPHDKTIIEDSAGASGLI
RNDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLF TDHLHGNTYGHAINFLRVDKHNPNAPVYLGFSSTSNV
GSLNEHFVMPESNI ANHQRFNKTPIKAVGSTKDYAQRVGTVSDTIAAIK GKVS SLENRLSAIHQEADIMA
AQAKVSQLQGLASTLTKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLASLKAALHQ
EALAEQAAARV TALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTSSLLNAQEALALQAKQSS
LEAT IATTEHQ LTLKTLANEKEYRHLDEDIATVPDLQVAPPLTG VKPLSYSKIDTTPLVQEMVKETKQLL
EASARLAAENTSLVAEALVGQ TSEMVA SNAIVSKITSSITQPSKTSYSGSSTT SNLISDVDESTQR

SEQ ID NO: 10 polynucleotide sequence encoding a fragment of GAS 40 with a C terminal
transmembrane sequence removed

ATGGACTTAGAACAAACGAAGCCAAACCAAGTTAAGCAGAAAATGCTTTAACCTCAACAATTGCTTTATT
GAGTGCCAGTGTAGGCGTATCTACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGCCGAGTA
ATACTCACGACGTAGTTTACCAAAC CAGAAAACAATCAAGAGGCAAAGGCAACTATTGATGCAGTTGAA
AAA ACTCTCAGTCAACAAAAGCAGAACTGACAGAGCTTGCTACCGCTCTGACAAAACTACTGCTGAAAT
CAACCACTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAATTTACACTAATA
CTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCTACTGAA
ACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACTGCATTTGTCAGAACAAAAGCTAGCAT
TTCAGCAGAAACTACTCGAGCTCAAGATTTAGTGGAACAAGTCAAACGCTCTGAACAAAATATTGCTAAGC
TCAATGCTATGATTAGCAATCC TGATGCTATCACTAAAGCAGCTCAAACGGCTAATGATAATACAAAAGCA
TTAAGCTCAGAAATGGAGAAGGCTAAAGCTGACTTAGAAAATGAAAAGCTAAAGTTAAAAGCAATTGAC
TGAAGAGTTGGCAGCTCAGAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAGTCTGCTTTAACTCTCAG
CTCCGCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAAGCACC GCAAGGCTATCTCTTGAAGAA
CTTAAAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTTATACAAAGAGCATGCAGA
TCAAATTTATGCCAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGATATTCAGCAGATCGTAATC
GCTTTGTTGATCCCGATAATTTGACACCAGAAGTGCAAATGAGCTAGCGCAGTTTGCAGCTCACATGATT
AATAGTGTAAGAAGACAATTAGGCTACCACCAGTTACTGTTACAGCAGGATCACAAGAATTTGCAAGATT
ACTTAGTACCAGCTATAAGAAACTCATGGTAATACAAGACCATCATTTGTCTACGGACAGCCAGGGGTAT
CAGGGCATTATGGTGTGGGCCCTCATGATAAACTATTATTGAAGACTCTGCCGGAGCGTCAGGGCTCATT
CGAAATGATGATAACATGTACGAGAATATCCGGTCTTTAACGATGTGCATAC TGTGAATGGTATTAAACG
TGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAATACATACGGCCATGCTA
TTAACTTTTACGTGTAGATAAACATAACCCTAATGCGCCTGTTTACCTTGGATTTTCAACCAGCAATGTA
GGATCTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACATTTGCTAACCATCAACGCTTTAATAAGAC
CCCTATAAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGTAGGCAC TGTATCTGATACTATTGCAG
CGATCAAAGGAAAAGTAAAGCTCATTAGAAAATCGTTTGTCCGCTATTCATCAAGAAGCTGATATTATGGCA
GCCAAAGCTAAAAGTAAAGTCAACTTCAAGGTAAATTAGCAAGCACACTTAAGCAGTCAGACAGCTTAAATCT
CCAAGTGAGACAATTAATGATAC TAAAGGTTCTTTGAGAACAGAAATTA CTAGCAGCTAAAGCAAAAACAG
CACAACCTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTGAAAGCCGCCTGCACCAGACA
GAAGCTTAGCAGAGCAAGCCGCAGCAGAGTGACAGCACTGGTGGCTAAAAAAGCTCATTGCAATATCT
AAGGGACTTTAAATGAATCC TAACCGCCTTCAAGTGATACGTGAGCGCATTGATAATACTAAGCAAGATT

SEQUENCE LISTING

TGGCTAAAACCTACCTCATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCTAAACAAAGCAGT
CTAGAAGCTACTATTGCTACCACAGAACACCAGTTGACTTTGCTTAAAACCTTAGCTAACGAAAAGGAATA
TCGCCACTTAGACGAAGATATAGCTACTGTGCCTGATTTGCAAGTAGCTCCACCTCTTACGGGCGTAAAAC
CGCTATCATATAGTAAGATAGATACTACTCCGCTTGTTCAGAAATGGTTAAAGAAACGAAACAACCTATTA
GAAGCTTCAGCAAGATTAGCTGCTGAAAATACAAGTCTTGTAGCAGAAGCGCTTGTGGCCAAACCTCTGA
AATGGTAGCAAGTAATGCCATTGTGTCTAAAATCACATCTTCGATTACTCAGCCCTCATCTAAGACATCTT
ATGGCTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTGATGAAAGTACTCAAAGA

SEQ ID NO: 11 amino acid sequence comprising a transmembrane region of GAS 40 as shown in Figures 1 and 2. ALKAGVVMLAAVGLTG

SEQ ID NO: 12 amino acid sequence comprising a first coiled-coil region of GAS 40
ETIQEAKATIDAVEKTL SQQKAE LTELALATKTTAEINHLKEQQDNEQKAL TSAQEIYTNNTLASSEETLL
AQGAHQRELTATETELHNAQADQHSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLNAMI SNPDA
ITKAAQTANDNTKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRLKSSA

SEQ ID NO: 13 amino acid sequence comprising a second coiled-coil region of GAS 40
RLSAIHQEADIMAAQKVSQ LQGLASTLKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSL
AKLASLKAALHQTEALAEQAAARVTALVAKKAHLQYL RDFKLNPNRLQVIRERIDNTKQDLAKTTSLLNA
QEALAAQAKQSSLEAT IATTEHQLTLLKTLANEKE

SEQ ID NO: 14 amino acid sequence comprising a leucine zipper motif within the second coiled-coil region of GAS 40.
QVIRERIDNTKQDLAKTTSLLNAQEALAAAL

SEQ ID NO: 15 amino acid sequence comprising SpA from *Streptococcus gordonii* Genbank reference GI 25990270

MNKRKEVFGFRKSKVAKTLCGAVLGAALIAIADQQVLADEVTETNSTANVAVTTTGNPATNLPEAQGEATE
AASQSQAQAGSKEGALPVEVSADDLNQAVTDAKAAGVNVVQDQTS DKGTATTAENAQKQAEIKSDYAKQA
EEIKKTTEAYKKEVEAHQAETDKINAENKAAEDKYQEDLKAHQAEVEKINTANATAKAEYEAKLAQYQKDL
AAVQKANEDSQLDYQNKLSAYQAELARVQKANAEAKEAYEKAVKENTAKNAALQAENEAIKQRNETAKANY
DAAMKQYEAADLAAIKKAKEDNDADYQAKLAAYQAELARVQKANADAKAAYEKAVEENTAKNTAIQAENEAI
KQRNAAKATYEAAALKQYEAADLAAKKNEDSDADYQAKLAAYQTELARVQKANADAKAAYEKAVEDNKAK
NAALQAENEEIKQRNAAKTDYEAKLAKYEADLAKYKKE LAEYPAKLKAYEDEQAQIKAAALVELEKNKNQD
GYLSKPSAQSLVYDSEPNALSLTTNGKMLKASAVDEAF SHDTAQYSKKILQPDNLNVSYLQQADDVTS SM
ELYGNFGDKAGWTTTVGNNTVEVKFASVLLERGSVTATYTNLEKSYNGKKISKAVFKYSLSDSKFKNVD
KAWLGVLPDPTLGVFASAYTGQEEKDTSIFIKNEFTFYDENDQPINFDNALLSVASLNRENNSIEMAKDYS
GTFVKISGSSVGEKDGKIYATETLNFKQGGSRWMTMYKNSQPGSGWSDSDAPNSWYGAGAISMSGPTNHV
TVGAI SATQVVP SDPVM AVATGKRPN IWIYSLNGKIRAVNVPKITKEKPTPPVAPTEPQAPTYEVEKPLEPA
PVAPTYENEPTPPVKTPDQPEPSKPEEPTYETEKPLEPAPVVPPTYENEPTPPVKTPDQPEPSKPEEPTYET
EKPLEPAPVAPTYENEPTPPVKTPDQPEPSKPEEPTYDPLPTPPVAPT PKQLPTPPVVPTVHFHYSSLLAQ
PQINKEIKNEDGVDIDRTLVAQSIKVFELKTEALTAGRPKTTSFVLVDPLPTGYKFDLDATKAASTGFDT
TYDEASHTVTFKATDETLATYNADLTKPVETLHP TVVGRV LNDGATYINNFTLTVNDAYGIKSNVVRV TTP
GKPNDPDNPNNNYIKPTKVNKNKEGLNIDGKEVLGASTNYELTWDL DQYKGDKSSKEAIQNGFYVDDYP
EEALDVRPDLVKVADEKGNQVSGVSVQYD SLEAAPKRVQDLLKANI TVKGA FQLFSADNPEEFYKQYVS
TGTSLVITDPM TVKSEFGKTGGKYENKAYQIDFGNGYATEVVVNNVPKITPKKDVTVSLDPTSENLDGQTV
QLYQTFNYRLIGGFIPQNHSEELDY SFVDDYDQAGDQYTGNYKTFSSLNLTMKDGSVIKAGTDLTSQTTA
ETDAANGIVTVRSKEDSLQKISLDS PFQAETYLQMRRIAIGTFENTYVNTV NKVAYASNTV RTTTTPIPRTP
DKPTPIPTPKPKDPKPKPETPKPKVSPKVEDPSAPIPVSVGKELTTLPKTGTNDSSYMPYLG LAAALVGV L
GLGQLKRKEDESN

SEQ ID NO: 16 amino acid sequence comprising Streptococcal surface protein B precursor from *Streptococcus gordonii* Genbank reference GI 25055226 AAC44102.3

SEQUENCE LISTING

MQKREVFGRKSKVAKTLCGAVLGAALIAIADQQVLADEVTTETNSTANVAVT'TTGNPATNLPEAQGEATEA
 ASQSQAQAGSKD GALPVEVSADDLNKAVTDAKAAGVNVVQDQTSKDGTA'TTAAENAQKQAEIKSDYAKQAE
 EIKKTTTEAYKKEVEAHQAE'TDKINAENKAAEDKYQEDLKAHQAEVEKINTANATAKA'EYEAQKLAQYQKDLA
 AVQKANEDSQLDYQNKLSAYQAE'LARVQKANAEAKEAYEKAVKENTAKNAALQAENEAIKQRNETAKANYD
 AAMKQY'EADLAAIKKAKEDNDADYQAKLAAYQAE'LARVQKANADAKAAAYEKAVEENTAKNTAIQAENEAIK
 QRNETAKATYEA'AVKQY'EADLAAVKQANATNEADYQAKLAAYQTE'LARVQKANADAKATYEKAVEDNKAKN
 AALQAENE'EKQRNAAAKTDYEA'KLAKYEADLAKYKKDFAAYTAALAEAESKKKQDGYLSEPRSQSLNFKS
 EPNAIRTI'DSSVHQY'GQQELDALVKS'WGI SPTN'PDRKKSTAYS'YFNAINSNNTYAKLVLEKDKPVDV'TYTG
 LKNSSFN'GKKISKV'VYTYTLKETGFDDG'TKMTMFASSDPTV'TAWYNDYFTSTNINVKVKFYDEEGQLMNL'T
 GGLVNF'SSLNRGNGSGAIDKDAI'ESVRN'FN'GRYIPI'SGSSIKI'HENNSAYADSSNAEKSRGARWDTSEWDT
 TSSPNNWYGAIVGEITQSEISFNMASSKSGNIWF'AFNSNINAI'GVPTKPVAPTAPTQPMYETEKPLEPAPV
 VPTYENEPT'PPVKTPDQPEPSKPEEPTYE'TEKPLEPAPVAPT'YENEPT'PVKIPDQPEPSKPEEPTYE'TEK
 PLEPAPVAPT'YENEPT'PVKTPDQPEPSKPEEPTYD'PLPTPPLAPT'PKQLPT'PPVPTVHFHYSSLLAQ'PQ
 INKEIK'NEDGVDIDR'TLVAKQSIGK'FELKTEALTAGR'PKTTSFVLVD'PLPTGYKFDL'DATKAASTGF'DTTY
 DEASHTV'TFKATDETLATYNADLTK'PVETLHPTV'VGRVLNDGATYTN'NFTLTVNDAYGIKSNVVR'TTPGK
 PNDPDN'PNNYIKPTKVNKNKEGLNIDG'KEVL'AGSTNY'YELTWDLDQYKGD'KSKEAIQNGFY'YVDDY'PEE
 ALDVRPDLV'KVADEKGNQVSGVSV'QQYDSLEA'APK'KVQDLLK'KANTVKGAFQLF'SADNPEEFYKQYV'STG
 TSLVITDPMTVKSEFGKTGGKYENKAYQID'FGNGYATEV'VNNV'PKITPKKDVTVSLDPTSENLDGQTVQL
 YQTFNYRLIGGFIPQNHSE'LEDYSFVDDYDQAGDQYTG'NYKTFSSLNLTMKDGSVIKAGTDLTSQTTAET
 DATNGIVTVRFKEDFLQKISLDS'PFQAETYLQ'RRRIAIGTF'ENTYVNTV'NKVAYASNTV'RTTTPIPRTPDK
 PTPIPTPKPKDPDKPETPKEPKVPS'PKVEDPSA'PIPVSVGKELTTL'PKTGTNDATYMPYLGLAALVGFGLG
 GLAKRKED

SEQ ID NO: 17 amino acid sequence comprising PspA from *Streptococcus pneumoniae*

Genbank reference GI 282335

MNKKKMIL'TSLASVA'ILGAGFVASQPT'VVRAEES'PVASQSKAEKDYDA'AKKDAKNAKKA'VEDAQKALDDAK
 AAQKKYDE'DQKKTEEKA'ALEKAASE'EMDKAVA'AVQQAYLAYQQATD'KAADKDAADKMID'EAKKREEEAKTKF
 NTVRAMV'VPEPEQLAET'KKKSEEAKQ'KAPELTKKLE'EKAKLEEA'EKKATEAKQ'KVDAEEVAPQAKIA'ELE
 NQVHRL'EQLKEID'ESSEDYAKE'GFRAPLQSKL'DAKKAKLSKLE'ELSDKIDELDAE'IAKLEDQLKAAE'EN
 NNVEDY'FKGLEKTIA'AKKAELEKTE'ADLKKAVNE'PEKPAPAPET'PAPEAPAEQPK'PAPAPQAPAPK'PEK
 PAEQPK'PEKTDQQA'EEDYARRSE'EEYNR'LTQQPPKAEK'PAPAPKTGWKQ'ENGMMWYFYNTD'GSMATGWLQ
 NNGSWY'YLN'NGAMATGWLQYNGS'WY'LNANGAMATG'WAKVNGSWY'YLNANGAMATG'WLQYNGS'WY'YLNAN
 GAMATG'WAKVNGS'WY'YLNANGAMATG'WLQYNGS'WY'YLNANGAMATG'WAKVNGS'WY'YLNANGAMATG'WVKD
 DTWY'YLEASGAMKASQ'WFKVSDK'WY'YVNGLGALAVNT'TVDGYKVNANG'EWV

SEQ ID NO: 18 amino acid sequence comprising a portion of Se89.9 of *Streptococcus equi*

Genbank reference GI 2330384

ESDIVDATR'FSTTEI'PKSGQVIDRS'ASIQAL'TNDIASIKGKIASLES'RLADPSSEAEV'TAAQAKISQLQH
 QLEAAQAKSHKLDQ'QVEQLANTK'DSLR'QLLAAKEEQ'ALKANLDKALALLASSKATLHKLEA'AMEEAKA
 RVAGLASQKAQLE'DLLAF'EKPNR'IELAQEKVAAAKKALADTE'DKLLAAQASLSDLQAQRARLQLSIATI

SEQ ID NO: 19 polynucleotide sequence comprising GST-40-HIS

CTGGTTCGCGTGGATCCCATATGAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGG
 AGAAACGAAGGCGAGTAATACTCACGACGATAGTTTACCAAAAACAGAAACAATTCAAGAGGCAAAGGCAA
 CTATTGATGCAGTTGAAAAACCTCAGTCAACAAAAGCAGAACTGACAGAGCTTGCTACCGCTCTGACA
 AAAACTACTGCTGAAATCAACCACCTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACA
 AGAAATTTACTACTAATACTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAG
 AGTTAACAGCTACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACTGCATTGTCA
 GAACAAAAAGCTAGCATTTTCAGCAGAACTACTCGAGCTCAAGATTTAGTGAACAAGTCAAACGCTCTGA
 ACAAATATTGCTAAGCTCAATGCTATGATTAGCAATCCTGATGCTATCACTAAAGCAGCTCAAACGGCTA
 ATGATAATACAAAAGCATTAAGCTCAGAATTGGAGAAGGCTAAAAGCTGACTTAGAAAATCAAAGCTAAA
 GTTAAAAAGCAATTGACTGAAGAGTTGGCAGCTCAGAAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAG
 TCGTCTTAAATCCTCAGCTCCGCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCAGCAAG
 GCTATCCTCTTGAAGAACTTAAAAAATTAGAAGTAGTGGTTATATTTGGATCAGCTAGTTACAATAATTAT
 TACAAAGAGCATGCAGATCAAATTTATGCCAAAGCTAGTCCAGGTAATCAATTAATCAATCAATCAAGATAT
 TCCAGCAGATCGTAATCGCTTTGTTGATCCCATAATTTGACACCAGAAGTGCAAAATGAGCTAGCGCAGT

SEQUENCE LISTING

TTGCAGCTCACATGATTAATAGTGTAAAGAAGACAATTAGGTCTACCACCAGTTACTGTTACAGCAGGATCA
CAAGAATTTGCAAGATTACTTAGTACCAGCTATAAGAAAACATCATGGTAATACAAGACCATCATTTTGTCTA
CGGACAGCCAGGGGTATCAGGGCATTATGGTGTGGGCTCATGATAAAACTATTATTGAAGACTCTGCCG
GAGCGTCAGGGCTCATTCGAAATGATGATAACATGTACGAGAATATCGGTGCTTTTAACGATGTGCATACT
GTGAATGGTATTAACCGTGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAAA
TACATACGGCCATGCTATTAACCTTTTACGTGTAGATAAACATAAACCTAATGCGCCTGTTTACCTTGGAT
TTTCAACCAGCAATGTAGGATCTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACATTGCTAACCAT
CAACGCTTTAATAAGACCCCTATAAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGTAGGCACTGT
ATCTGATACTATTGACAGCGATCAAAGGAAAAGTAAGCTCATTAGAAAAATCGTTTGTCCGGCTATTCATCAAG
AAGCTGATATTTATGGCAGCCCAAGCTAAAGTAAGTCAACTTCAAGGTAAATTAGCAAGCACACTTAAGCAG
TCAGACAGCTTAAATCTCCAAGTGAGACAATTAATGATACTAAAGGTTCTTTGAGAACAGAATTACTAGC
AGCTAAAGCAAAACAAGCACAACCTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTGAAAG
CCGCACTGCACCAGACAGAAGCCTTAGCAGAGCAAGCCGAGCCAGAGTGACAGCACTGGTGGCTAAAAAA
GCTCATTTGCAATATCTAAGGGACTTTAAATTTGAATCCTAACCGCCTTCAAGTGATACGTGAGCGCATTGA
TAATACTAAGCAAGATTTGGCTAAAACCTACCTCATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTAC
AAGCTAAACAAGCAGTCTAGAAGCTACTATTGCTACCACAGAACCAGTTGACTTTGCTTAAAACCTTA
GCTAACGAAAAGGAATATCGCCACTTAGACGAAGATATAGCTACTGTGCCTGATTTGCAAGTAGCTCCACC
TCTTACGGGCGTAAAACCGCTATCATATAGTAAGATAGATACTACTCCGCTTGTTCAGAAATGGTTAAAG
AAACGAAAACAACATTAGAAAGCTTTCAGCAAGATTAGCTGCTGAAAATACAAGTCTTGTAGCAGAAGCGCTT
GTTGGCCAAACCTCTGAAATGGTAGCAAGTAATGCCATTGTGTCTAAAATCACATCTTCGATTACTCAGCC
CTCATCTAAGACATCTTATGGCTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTGATGAAAGTA
CTCAAAGAGCTCTTAAAGCAGGAGTCTGTCATGTTGGCAGCTGTCCGGCCTCACAGGATTTAGGTTCCGTAAG
GAATCTAAGCGCGCCGCACTCGAGCACCACCACCACCACCACC

SEQ ID NO: 20 amino acid sequence comprising GST-40-HIS

L V P R G S H Met S V G V S H Q V K A D D R A S G E T K A S N T H D D S
L P K P E T I Q E A K A T I D A V E K T L S Q Q K A E L T E L A T A L T
K T T A E I N H L K E Q Q D N E Q K A L T S A Q E I Y T N T L A S S E E
T L L A Q G A E H Q R E L T A T E T E L H N A Q A D Q H S K E T A L S E
Q K A S I S A E T T R A Q D L V E Q V K T S E Q N I A K L N A Met I S N
P D A I T K A A Q T A N D N T K A L S S E L E K A K A D L E N Q K A K V
K K Q L T E E L A A Q K A A L A E K E A E L S R L K S S A P S T Q D S I
V G N N T Met K A P Q G Y P L E E L K K L E A S G Y I G S A S Y N N Y Y
K E H A D Q I I A K A S P G N Q L N Q Y Q D I P A D R N R F V D P D N L
T P E V Q N E L A Q F A A H Met I N S V R R Q L G L P P V T V T A G S Q
E F A R L L S T S Y K K T H G N T R P S F V Y G Q P G V S G H Y G V G P
H D K T I I E D S A G A S G L I R N D D N Met Y E N I G A F N D V H T V
N G I K R G I Y D S I K Y Met L F T D H L H G N T Y G H A I N F L R V D
K H N P N A P V Y L G F S T S N V G S L N E H F V Met F P E S N I A N H
Q R F N K T P I K A V G S T K D Y A Q R V G T V S D T I A A I K G K V S
S L E N R L S A I H Q E A D I Met A A Q A K V S Q L Q G K L A S T L K Q
S D S L N L Q V R Q L N D T K G S L R T E L L A A K A K Q A Q L E A T R
D Q S L A K L A S L K A A L H Q T E A L A E Q A A A R V T A L V A K K A
H L Q Y L R D F K L N P N R L Q V I R E R I D N T K Q D L A K T T S S L
L N A Q E A L A A L Q A K Q S S L E A T I A T T E H Q L T L L K T L A N
E K E Y R H L D E D I A T V P D L Q V A P P L T G V K P L S Y S K I D T
T P L V Q E Met V K E T K Q L L E A S A R L A A E N T S L V A E A L V G
Q T S E Met V A S N A I V S K I T S S I T Q P S S K T S Y G S G S S T T
S N L I S D V D E S T Q R A L K A G V V Met L A A V G L T G F R F R K E
S K A A A L E H H H H H H

SEQ ID NO: 21 polynucleotide sequence comprising 40a-HIS

ATGAGTGTAGGCGTATCTACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGCGAGTAATAC
TCACGACGATAGTTTACCAAAACCAGAAACAATTCAAGAGGCAAAGGCAACTATTGATGCAGTTGAAAAAA
CTCTCAGTCAACAAAAAGCAGAAGCTGACAGAGCTTGCTACCGCTCTGACAAAAACTACTGCTGAAATCAAC
CACTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAATTTACTACTAATACTCT

SEQUENCE LISTING

TGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCTACTGAAACAG
AGCTTCATAATGCTCAAGCAGATCAACATTCAAAGAGACTGCATTGTCAGAACAAAAAGCTAGCATTTC
GCAGAACTACTCGAGCTCAAGATTTAGTGGAAACAAGTCAAACCGTCTGAACAAAAATATTGCTAAGCTCAA
TGCTATGATTAGCAATCTGATGCTATCAGTAAAGCAGCTCAAACGGCTAATGATAATACAAAAGCATTAA
GCTCAGAATTGGAGAAGGC TAAAGCTGACTTAGAAAATCAAAAAAGCTAAAGTTAAAAAGCAATTGACTGAA
GAGTTGGCAGCTCAGAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAGTCGTCTTAAATCCTCAGCTCC
GTCTACTTAAGATAGCATTGTGGGTAATAATACCATGAAAGACCCGCAAGGCTATCCTCTTGAAGAACTTA
AAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAAATTATFACAAAGAGCATGCAGATCAA
ATTATTGCCAAAGCTAGTCCAGGTAATCAATTAAATCAATACCAAGATATTCCAGCAGATCGTAATCGCTT
TGTTGATCCCATAATTTGACACCAGAAGTGCAAAATGAGCTAGCGCAGTTTGCAGCTCACATGATTAATA
GTGTAAGAAGACAATTAGGCTACCACCAGTTACTGTTACAGCAGGATCACAAGAATTTGCAAGATTACTT
AGTACCAGCTATAAGAAAACTCATGGTAATACAAGACCATCATTTGTCTACGGACAGCCAGGGGTATCAGG
GCATTATGGTGTGGGCCCTCATGATAAAACTATTATTGAAGACTCTGCCGGAGCGTCAGGGCTCATTTCGAA
ATGATGATAACATGTACGAGAATATCGGTGCTTTTAAACGATGTGCATACTGTGAATGGTATTAACGTGGT
ATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAAATACATACGGCCATGTATTAA
CTTTTTACGTGTAGATAAAACATAACCCTAATGCGCCTGTTTACCTTGGATTTTCAACCAGCAATGTAGGAT
CTTTGAATGAACACTTTGTAAATGTTTCCAGAGTCTAACATTGCTAACCATCAACGCTTTAATAAGACCCCT
ATAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGTAGGCACTGTATCTGATAC TATTGCAGCGAT
CAAAGGAAAAAGTAAAGCTCATTAGAAAATCGTTTGTGCGCTATTTCATCAAGAAGCTGATATTATGGCAGCCC
AAGCTAAAAGTAAAGTCAACTTCAAGGTAAATTAGCAAGCACACTTAAGCAGTCAGACAGCTTAAATCTCCAA
GTGAGACAATTAAATGATAC TAAAGGTTCTTTGAGAACAGAATTACTAGCAGCTAAAGCAAAAACAAGCACA
ACTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTGAAAAGCCGCCTGCACCAGACAGAAG
CCTTAGCAGAGCAAGCCGCAGCCAGAGTGACAGCACTGGTGGCTAAAAAAGCTCATTTGCAATATCTAAGG
GACTTTAAATGAAATCCTAACCGCCTTCAAGTGATACGTGAGCGCATTGATAATACTAAGCAAGATTTGGC
TAAAACTACCTCATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCTAAACAAGCAGCTAG
AAGTACTATTGCTACCACAGAACACCAGTTGACTTTGCTTAAAACCTTAGCTAACGAAAAGGAATATCGC
CACTTAGACGAAGATATAGCTACTGTGCCTGATTTGCAAGTAGCTCCACCTCTTACGGGCGTAAAACCGCT
ATCATATAGTAAGATAGATACTACTCCGCTTGTTCAGAAAATGGTTAAAGAAACGAAACAAC TATTAGAAG
CTTCAGCAAGATTAGCTGCTGAAAATACAAGTCTTGTAGCAGAAGCGCTTGTGGCCAAACCTCTGAAATG
GTAGCAAGTAATGCCATTGTGTCTAAAATCACATCTTCGATTACTCAGCCCTCATCTAAGACATCTTATGG
CTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTGATGAAAAGTACTCAAAGTGGCGGCGCACTCG
AGCACCACCACCACCACCAC

SEQ ID NO: 22 amino acid sequence comprising 40a-HIS

M S V G V S H Q V K A D D R A S G E T K A S N T H D D S L P K P E T I Q
E A K A T I D A V E K T L S Q Q K A E L T E L A T A L T K T T A E I N H
L K E Q Q D N E Q K A L T S A Q E I Y T N T L A S S E E T L L A Q G A E
H Q R E L T A T E T E L H N A Q A D Q H S K E T A L S E Q K A S I S A E
T T R A Q D L V E Q V K T S E Q N I A K L N A Met I S N P D A I T K A A
Q T A N D N T K A L S S E L E K A K A D L E N Q K A K V K K Q L T E E L
A A Q K A A L A E K E A E L S R L K S S A P S T Q D S I V G N N T M K A
P Q G Y P L E E L K K L E A S G Y I G S A S Y N N Y Y K E H A D Q I I A
K A S P G N Q L N Q Y Q D I P A D R N R F V D P D N L T P E V Q N E L A
Q F A A H M I N S V R R Q L G L P V T V T A G S Q E F A R L L S T S Y
K K T H G N T R P S F V Y G Q P G V S G H Y G V G P H D K T I I E D S A
G A S G L I R N D D N M Y E N I G A F N D V H T V N G I K R G I Y D S I
K Y M L F T D H L H G N T Y G H A I N F L R V D K H N P N A P V Y L G F
S T S N V G S L N E H F V M F P E S N I A N H Q R F N K T P I K A V G S
T K D Y A Q R V G T V S D T I A A I K G K V S S L E N R L S A I H Q E A
D I M A A Q A K V S Q L Q G K L A S T L K Q S D S L N L Q V R Q L N D T
K G S L R T E L L A A A K A K Q A Q L E A T R D Q S L A K L A S L K N A L
H Q T E R A L E Q A A A R V T A L V A K K A H L Q Y L R D F K L N P N R
L Q V I R E R I D N T K Q D L A K T T S S L L N A Q E A L A A L Q A K Q
S S L E A T I A T T E H Q L T L L K T L A N E K E Y R H L D E D I A T V
P D L Q V A P P L T G V K P L S Y S K I D T T P L V Q E M V K E T K Q L
L E A S A R L A A E N T S L V A E A L V G Q T S E M V A S N A I V S K I

SEQUENCE LISTING

T S S I T Q P S S K T S Y G S G S S T T S N L I S D V D E S T Q R A A A
L E H H H H H H H

SEQ ID NO: 23 polynucleotide sequence comprising 40a-RR-HIS

ATGAGTGTAGGCCTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGGCGAGTAATAC
TCACGACGATAGTTTACCAAACCAGAAACAATTCAGAGGCCAAAGGCAACTATTGATGCAGTTGAAAAAA
CTCTCAGTCAACAAAAAGCAGAAGTACAGAGCTTGCATCCGCTCTGCACAAAACACTACTGCTGAAATCAAC
CACTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAATTTACTACTAATACTCT
TGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCTACTGAAAACAG
AGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACTGCATTTGTCAGAACAAAAGCTAGCATTTC
GCAGAACTACTCGAGCTCAAGATTTAGTGGAAACAAGTCAAACGCTCTGAACAAAATATTGCTAAGCTCAA
TGCTATGATTAGCAATCCTGATGCTATCCTAAAGCAGCTCAAACGGCTAATGATAATACAAAAGCATTAA
GCTCAGAAATGGAGAAGGCTAAAGCTGACTTAGAAAATCAAAAAGCTAAAGTTAAAAAGCAATTGACTGAA
GAGTTGGCAGCTCAGAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAGTTCGTCTTAAATCCTCAGCTCC
GTCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCAGGCAAGGCTATCCTCTTGAAGAACTTA
AAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACAAGAGCATGCAGATCAA
ATTATTGCCAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGATATTCCAGCAGATCGTAATCGCTT
TGTTGATCCCGATAATTTGACACCAGAAGTGAAAATGAGCTAGCGCAGTTTGCAGCTCACATGATTAATA
GTGTAcGtGtCAATTAGGTCTACCACCAGTTACTGTTACAGCAGGATCACAAGAATTTGCAAGATTACTT
AGTACCAGCTATAAGAAAACCTCATGGTAATACAAGACCATCATTTGCTTACGGACAGCCAGGGGTATCAGG
GCATTATGGTGTGGGCCCTCATGATAAAACTATTATTGAAGACTCTGCCGGAGCGTCAGGGCTCATTTCGAA
ATGATGATAACATGTACGAGAATATCGGTGCTTTTAAACGATGTGCATACGTGAATGGTATTAAACGTGGT
ATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAAATACATACGGCCATGCTATTAA
CTTTTACGTGTAGATAAACATAACCTTAATGCGCCTGTTTACCTTGGATTTTCAACCAGCAATGTAGGAT
CTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACATTGCTAACCATCAACGCTTTAATAAGACCCCT
ATAAAAAGCCGTTGGAAGTACAAAAGATTATGCCAAAAGAGTAGGCACGTGTATCTGATACTATTGCAGCGAT
CAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTTGTCGGCTATTCATCAAGAAGCTGATATTATGGCAGCCC
AAGCTAAAGTAAGTCAACTTCAAGGTAATTAGCAAGCACACTTAAAGCAGTCAGACAGCTTAAATCTCCAA
GTGAGACAATTAATGATACTAAAGGTTCTTTGAGAACAGAATTACTAGCAGCTAAAGCAAACAAGCACA
ACTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTGAAAGCCGCACTGCACCAGACAGAAG
CCTTAGCAGAGCAAGCCGACAGGAGTGACAGCACTGGTGGCTAAAAAAGCTCATTGCAATATCTAAGG
GACTTTAAATGAAATCCTAACCGCCTTCAAGTACGCTGAGCGCATTTGATAATACTAAGCAAGATTTGGC
TAAACTACTCTATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCTAAACAAGCAGTCTAG
AAGCTACTATTGCTACCACAGAACCAGTTGACTTTGCTTAAAACCTTAGCTAACGAAAAGGAATATCGC
CACTTAGACGAAGATATAGCTACTGTGCCTGATTTGCAAGTAGCTCCACCTCTTACGGGCGTAAAACCGCT
ATCATATAGTAAGATAGATACTACTCCGCTTGTTCAGAAAATGGTTAAAGAAACGAAACAACCTATTAGAAG
CTTCAGCAAGATTAGCTGCTGAAAATACAAGTCTTGTAGCAGAAGCGCTTGTGGCCAAACCTCTGAAATG
GTAGCAAGTAATGCCATTGTGTCTAAAATCACATCTTCGATTACTCAGCCCTCATCTAAGACATCTTATGG
CTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTGATGAAAGTACTCAAAGTGGCGCCGCACTCG
AGCACCACCACCACCACCACC

SEQ ID NO: 24 amino acid sequence comprising 40a-RR-HIS

M S V G V S H Q V K A D D R A S G E T K A S N T H D D S L P K P E T I Q
E A K A T I D A V E K T L S Q Q K A E L T E L A T A L T K T T A E I N H
L K E Q Q D N E Q K A L T S A Q E I Y T N T L A S S E E T L L A Q G A E
H Q R E L T A T E T E L H N A Q A D Q H S K E T A L S E Q K A S I S A E
T T R A Q D L V E Q V K T S E Q N I A K L N A Met I S N P D A I T K A A
Q T A N D N T K A L S S E L E K A K A D L E N Q K A K V K K Q L T E E L
A A Q K A A L A E K E A E L S R L K S S A P S T Q D S I V G N N T M K A
P Q G Y P L E E L K K L E A S G Y I G S A S Y N N Y Y K E H A D Q I I A
K A S P G N Q L N Q Y Q D I P A D R N R F V D P D N L T P E V Q N E L A
Q F A A H M I N S V R R Q L G L P P V T V T A G S Q E F A R L L S T S Y
K K T H G N T R P S F V Y G Q P G V S G H Y G V G P H D K T I I E D S A
G A S G L I R N D D N M Y E N I G A F N D V H T V N G I K R G I Y D S I
K Y M L F T D H L H G N T Y G H A I N F L R V D K H N P N A P V Y L G F
S T S N V G S L N E H F V M F P E S N I A N H Q R F N K T P I K A V G S

SEQUENCE LISTING

T K D Y A Q R V G T V S D T I A A I K G K V S S L E N R L S A I H Q E A
D I M A A Q A K V S Q L Q G K L A S T L K Q S D S L N L Q V R Q L N D T
K G S L R T E L L A A K A K Q A Q L E A T R D Q S L A K L A S L K A A L
H Q T E A L A E Q A A A R V T A L V A K K A H L Q Y L R D F K L N P N R
L Q V I R E R I D N T K Q D L A K T T S S L L N A Q E A L A A L Q A K Q
S S L E A T I A T T E H Q L T L L K T L A N E K E Y R H L D E D I A T V
P D L Q V A P P L T G V K P L S Y S K I D T P L V Q E M V K E T K Q L
L E A S A R L A A E N T S L V A E A L V G Q T S E M V A S N A I V S K I
T S S I T Q P S S K T S Y G S G S S T T S N L I S D V D E S T Q R A A A
L E H H H H H H H H

SEQ ID NO: 25 polynucleotide sequence comprising 40a-RR (nat)

ATGAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAAACGAAGGCGAGTAATAC
TCACGACGATAGTTTACCAAAACCAGAAACAATTCAAGAGGCCAAAGGCAACTATTGATGCAGTTGAAAAA
CTCTCAGTCAACAAAAAGCAGAACTGACAGAGCTTGCTACCGCTCTGACAAAAACTACTGCCTGAAATCAAC
CACTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAATTTACACTAATACTCT
TGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCTACTGAAACAG
AGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACTGCATTGTCAGAACA AAAAGCTAGCATTTCA
GCAGAACTACTCGAGCTCAAGATTTAGTGGAAACAAGTCAAACCGTCTGAACAAAATATTGCTAAGCTCAA
TGCTATGATTAGCAATCCTGATGCATCACTAAAGCAGCTCAAACGGCTAATGATAATACAAAAGCATTAA
GCTCAGAATTGGAGAAGGCTAAAGCTGACTTAGAAAATCAAAAAGCTAAAGTTAAAAAGCAATTGACTGAA
GAGTTGGCAGCTCAGAAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAGTCGCTCTTAAATCCTCAGCTCC
GTCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCGCAAGGCTATCCCTCTTGAAGAACTTA
AAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATATTACAAAAGAGCATGCAGATCAA
ATTATTGCCAAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGATATTCCAGCAGATCGTAATCGCTT
TGTTGATCCCATAATTTGACACCAGAAGTGCAAAATGAGCTAGCGCAGTTTGCAGCTCACATGATTAATA
GTGTACGtCgTCAATTAGGTCACCACCAGTTACTGTTACAGCAGGATCACAGAATTTGCAAGATTACTT
AGTACCAGCTATAAGAAAACTCATGGTAATACAAGACCATCATTTGTCTACGGACAGCCAGGGGTATCAGG
GCATTATGGTGTGGGCCCTCATGATAAAACTATTATTGAAGACTCTGCCGGAGCGTCAGGGCTCATTTCGAA
ATGATGATAACATGTACGAGAATATCGGTGCTTTTAAACGATGTGCATACTGTGAATGGTATTTAAACGTGGT
ATTTATGACGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAAATACATACGGCCATGCTATTAA
CTTTTGAACGTAGATAAAACATAACCCTAATGCGCCTGTTTACCTTGGATTTTCAACCAGCAATGTAGGAT
CTTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACACTTGCTAACCATCAACGCTTTTAATAAGACCCCT
ATAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGTAGGCACTGTATCTGATACTATTGCAGCGAT
CAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTGTGCGCTATTTCATCAAGAAGCTGATATTATGGCAGCCC
AAGCTAAAGTAAGTCAACTTCAAGGTAAATTAGCAAGCACACTTAAGCAGTCAGACAGCTTAAATCTCCAA
GTGAGACAATTAATGATACTAAAGGTTCTTTGAGAACAGAATTACTAGCAGCTAAAGCAAACAAGCACA
ACTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTGAAAAGCCGCCTGCACCAGACAGAAG
CCTTAGCAGAGCAAGCCGCAGCCAGAGTGACAGCACTGGTGGCTAAAAAAGCTCATTGCAATATCTAAGG
GACTTTAAATGAAATCCTAACCGCCTTCAAGTGATACGTGAGCGCATTTGATAATACTAAGCAAGATTTGGC
TAAAACCTACCTCATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCTAAACAAAGCAGTCTAG
AAGCTACTATTGCTACCACAGAACACCAGTTGACTTTGCTTAAAACCTTAGCTAACGAAAAGGAATATCGC
CACTTAGACGAAGATATAGCTACTGTGCCCTGATTTGCAAGTAGCTCCACCTCTTACGGGCGTAAAACCGCT
ATCATATAGTAAGATAGATACTACTCCGCTTGTTCAGAAATGGTTAAAAGAAACGAAACAACCTATTAGAAG
CTTCAGCAAGATTAGCTGCTGAAAATACAAGTCTTGTAGCAGAAGCGCTTGTGGCCAAACCTCTGAAATG
GTAGCAAGTAATGCCATTGTGCTAAAATCACATCTTCGATTACTCAGCCCTCATCTAAGACATCTTATGG
CTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTGATGAAAAGTACTCAACgT

SEQ ID NO: 26 amino acid sequence comprising 40a-RR (nat)

M S V G V S H Q V K A D D R A S G E T K A S N T H D D S L P K P E T I Q
E A K A T I D A V E K T L S Q Q K A E L T E L A T A L T K T T A E I N H
L K E Q Q D N E Q K A L T S A Q E I Y T N T L A S S E E T L L A Q G A E
H Q R E L T A T E T E L H N A Q A D Q H S K E T A L S E Q K A S I S A E
T T R A Q D L V E Q V K T S E Q N I A K L N A M I S N P D A I T K A A Q
T A N D N T K A L S S E L E K A K A D L E N Q K A K V K K Q L T E E L A
A Q K A A L A E K E A E L S R L K S S A P S T Q D S I V G N N T M K A P

SEQUENCE LISTING

Q G Y P L E E L K K L E A S G Y I G S A S Y N N Y Y K E H A D Q I I A K
A S P G N Q L N Q Y Q D I P A D R N R F V D P D N L T P E V Q N E L A Q
F A A H M I N S V R R Q L G L P P V T V T A G S Q E F A R L L S T S Y K
K T H G N T R P S F V Y G Q P G V S G H Y G V G P H D K T I I E D S A G
A S G L I R N D D N M Y E N I G A F N D V H T V N G I K R G I Y D S I K
Y M L F T D H L H G N T Y G H A I N F L R V D K H N P N A P V Y L G F S
T S N V G S L N E H F V M F P E S N I A N H Q R F N K T P I K A V G S T
K D Y A Q R V G T V S D T I A A I K G K V S S L E N R L S A I H Q E A D
I M A A Q A K V S Q L Q G K L A S T L K Q S D S L N L Q V R Q L N D T K
G S L R T E L L A A K A K Q A Q L E A T R D Q S L A K L A S L K A A L H
Q T E A L A E Q A A A R V T A L V A K K A H L Q Y L R D F K L N P N R L
Q V I R E R I D N T K Q D L A K T T S S L L N A Q E A L A A L Q A K Q S
S L E A T I A T T E H Q L T L L K T L A N E K E Y R H L D E D I A T V P
D L Q V A P P L T G V K P L S Y S K I D T T P L V Q E M V K E T K Q L L
E A S A R L A A E N T S L V A E A L V G Q T S E M V A S N A I V S K I T
S S I T Q P S S K T S Y G S G S S T T S N L I S D V D E S T Q R

SEQ ID NO: 27 polynucleotide sequence comprising HIS-40a NH

ATGGGATCGCATCACCATCACCATCAGCCTAGTAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAG
AGCCTCAGGAGAAACGAAGGCGAGTAATACTCAGCAGATAGTTTACCAAACCAGAAACAATTCAGAGG
CAAAGGCAACTATTGATGCAGTTGAAAAAAGCTCTCAGTCAACAAAAAGCAGAACTGACAGAGCTTGCTACC
GCTCTGACAAAAAAGTACTGCTGAAATCAACCACTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAAC
CTCTGCACAGAAATTTACACTAATACTCTTGAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAAC
AGCAAAGAGAGTTAACAGCTACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACT
GCATTGTCAGAACAAAAAGCTAGCATTTTCAGCAGAAACTACTCGAGCTCAAGATTTAGTGGAAACAAGTCAA
AACGCTGAAACAAAAATATTGCTAAGCTCAATGCTATGATTAGCAATCCTGATGCTATCACATAAGCAGCTC
AAACGGCTAATGATAATACAAAAGCATTAAGCTCAGAATTGGAGAAGGCTAAAGCTGACTTAGAAAATCAA
AAAGCTAAAGTTAAAAAGCAATTGACTGAAGAGTTGGCAGCTCAGAAAAGCTGCTCTAGCAGAAAAAGAGGC
AGAAGCTTAGTCGTCTTAAATCCTCAGCTCCGCTACTCAAGATAGCATTTGTGGGTAATAATACCATGAAAAG
CACCGCAAGGCTATCCTCTTGAAGAAGCTTAAAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTAC
AATAATTATTACAAAAGAGCATGCAGATCAAATTTATGCCAAAAGCTAGTCCAGGTAATCAATTAATCAATA
CCAAGATATTCCAGCAGATCGTAATCGCTTTGTTGATCCCGATAAATTTGACACCAGAAGTGCAAAATGAGC
TAGCGCAGTTTGCAGCTCACATGATTAATAGTAAAGAAGACAATTAGGTTACCACCAGTTACTGTGTTACA
GCAGGATCACAAGAATTTGCAAGATTACTTAGTACCAGCTATAAGAAAACATCATGGTAATACAAGACCATC
ATTTGTCTACGGACAGCCAGGGGTATCAGGGCATTATGGTGTGGGCCCTCATGATAAACTATTATTGAAG
ACTCTGCCGGAGCGTCAGGGCTCATTCGAAATGATGATAACATGTACGAGAATATCGGTGCTTTTAAACGAT
GTGCATACTGTGAATGGTATTAAACGTGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTT
ACACGGAAATACATACGGCCATGCTATTAACTTTTTACGTGTAGATAAACATAACCCATAATGCGCCTGTTT
ACCTTGGATTTTCAACCAGCAATGTAGGATCTTTGAATGAACACTTTGTAAATGTTTCCAGAGTCTAACATT
GCTAACCATCAACGCTTTAATAAGACCCCTATAAAAAGCCGTTGGAAGTACAAAAGACTATGCCCAAAGAGT
AGGCATGTATCTGATACTATTGCAGCGATCAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTGTGCGGCTA
TTTCATCAAGAAGCTGATATTTATGGCAGCCCAAGCTAAAGTAAGTCAACTTCAAGGTAATTAGCAAGCACA
CTTAAGCAGTCAGACAGCTTAAATCTCCAAGTGAGACAATTAATGATACTAAAGGTTCTTTGAGAACAGA
ATTACTAGCAGCTAAAGCAAAAACAAGCACAACCTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCAT
CGTTGAAAAGCCGCACTGCACCAGACAGAAGCCTTAGCAGAGCAAGCCGACAGCCAGAGTGACAGCACTGGTG
GCTAAAAAAGCTCATTGCAATATCTAAGGGACTTTAAATTTGAATCCTAACCGCCTTCAAGTGATACGTGA
GCGCATTGATAATACTAAGCAAGATTTGGCTAAAACCTCATCTTTGTTAAATGCACAAGAAGCTTTAG
CAGCCTTACAAGCTAAAACAAAGCAGTCTAGAAGCTACTATTGCTACCACAGAACCAGTTGACTTTGCTT
AAAACCTTAGCTAACGAAAAGGAATATCGCCACTTAGACGAAGATATAGCTACTGTGCCTGATTTGCAAGT
AGCTCCACCTTACGGGCGTAAAACCGCTATCATATAGTAAGATAGATACTACTCCGCTTGTTCAAGAAA
TGGTTAAAGAAAACGAAAACAATAATTAGAAGCTTCAGCAAGATAGCTGCTGAAAATACAAGTCTTTGATGCA
GAAGCGCTTGTGGCCAAAACCTCTGAAATGGTAGCAAGTAATGCCATTGTGCTTAAAATCACATCTTCGAT
TACTCAGCCCTCATCTAAGACATCTTATGGCTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTG
ATGAAAGTACTCAACcGt

SEQ ID NO: 28 amino acid sequence comprising HIS-40a NH

SEQUENCE LISTING

M G S H H H H H H A S S V G V S H Q V K A D D R A S G E T K A S N T H D
D S L P K P E T I Q E A K A T I D A V E K T L S Q Q K A E L T E L A T A
L T K T T A E I N H L K E Q Q D N E Q K A L T S A Q E I Y T N T L A S S
E E T L L A Q G A E H Q R E L T A T E T E L H N A Q A D Q H S K E T A L
S E Q K A S I S A E T T R A Q D L V E Q V K T S E Q N I A K L N A M I S
N P D A I T K A A Q T A N D N T K A L S S E L E K A K A D L E N Q K A K
V K K Q L T E E L A A Q K A A L A E K E A E L S R L K S S A P S T Q D S
I V G N N T M K A P Q G Y P L E E L K K L E A S G Y I G S A S Y N N Y Y
K E H A D Q I I A K A S P G N Q L N Q Y Q D I P A D R N R F V D P D N L
T P E V Q N E L A Q F A A H M I N S V R R Q L G L P P V T V T A G S Q E
F A R L L S T S Y K K T H G N T R P S F V Y G Q P G V S G H Y G V G P H
D K T I I E D S A G A S G L I R N D D N M Y E N I G A F N D V H T V N G
I K R G I Y D S I K Y M L F T D H L H G N T Y G H A I N F L R V D K H N
P N A P V Y L G F S T S N V G S L N E H F V M F P E S N I A N H Q R F N
K T P I K A V G S T K D Y A A Q R V G T V S D T I A A I K G K V S S L E N
R L S A I H Q E A D I M A A Q A K V S Q L Q G K L A S T L K Q S D S L N
L Q V R Q L N D T K G S L R T E L L A A K A K Q A Q L E A T R D Q S L A
K L A S L K A A L H Q T E A L A E Q A A A R V T A L V A K K A H L Q Y L
R D F K L N P N R L Q V I R E R I D N T K Q D L A K T T S S L L N A Q E
A L A A L Q A K Q S S L E A T I A T T E H Q L T L L K T L A N E K E Y R
H L D E D I A T V P D L Q V A P P L T G V K P L S Y S K I D T T P L V Q
E M V K E T K Q L L E A S A R L A A E N T S L V A E A L V G Q T S E M V
A S N A I V S K I T S S I T Q P S S K T S Y G S G S S T T S N L I S D V
D E S T Q R

SEQ ID NO: 29 polynucleotide sequence comprising HIS-40a CH

ATGGCTAGTAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGCGAG
TAATACTCACGACGATAGTTTACCAAACCAGAAAACAATTCAAGAGGCAAAGGCAACTATTGATGCAGTTG
AAAAAACTCTCAGTCAACAAAAAGCAGAAGCTGACAGAGCTTGTACCGCTCTGACGAAAACACTTGCTGAA
ATCAACCATTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAATTTACACTAA
TACTCTTGCAAGTAGTGAGGAGACGCTATTTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCTACTG
AAACAGAGCTTCATAATGCTCAAGCAGATCAACATTTCAAAGAGACTGCATTTGTCAGAACAAAAAGCTAGC
ATTTCAGCAGAAAACACTCGAGCTCAAGATTTAGTGGAAACAAGTCAAAAACGCTGTAACAAAATATTGCTAA
GCTCAATGCTATGATTAGCAATCCTGATGCTATCACTAAAGCAGCTCAAACGGCTAATGATAATACAAAAG
CATTAAAGCTCAGAAATGGGAGAAGGCTAAAGCTGACTTAGAAAATCAAAGCTAAAGTTAAAAGCAATTG
ACTGAAGAGTTGGCAGCTCAGAAAAGCTGCTCTAGCAGAAAAGAGGCAGAACTTAGTCGTCTTAAATCCTC
AGCTCCGTCTACTCAAGATAGCATTTGTGGGTAATAATACCATGAAAGCACCGCAAGGCTATCCTCTTGAAG
AACTTAAAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACAAAGAGCATGCA
GATCAAATTTATGCCAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGATATTCCAGCAGATCGTAA
TCGCTTTGTTGATCCCATAATTTGACACCAGAAGTGCAAAATGAGCTAGCGCAGTTTGCAGCTCACATGA
TTAATAGTGTAAGAAGACAATTAGGCTTACCACCAGTTACTGTTACAGCAGGATCACAAGAATTTGCAAGA
TTACTTAGTACCAGCTATAAGAAAACCTCATGGTAATACAAGACCATCATCTGCTACGGACAGCCAGGGGT
ATCAGGGCATTATGGTGTGGGCCCTCATGATAAAACTATTATTGAAGACTCTGCCGGAGCGTCAAGGGCTCA
TTCGAAATGATGATAACATGTACGAGAATATCGGTGCTTTTAAACGATGTGCATACGTGTAATGGTATTTAAA
CGTGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTACACGGAAATACATACGGCCATGC
TATTAACCTTTTACGTGTAGATAAACATAACCCTAATGCGCCTGTTTACCTTGGATTTTCAACCAGCAATG
TAGGATCTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACATTTGCTAACCATCAACGCTTTAATAAG
ACCCCTATAAAAAGCCGTTGGAGTACAAAAGATTATGCCCAAAGAGTAGGCACTGTATCTGATACTATTGC
AGCGTCAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTGTCCGGCTATTCATCAAGAAGCTGATATTTATGG
CAGCCCAAGCTAAAAGTAAGTCAACTTCAAGGTAATTTAGCAAGCACACTTAAGCAGTCAGACAGCTTAAAT
CTCCAAGTGAGACAATFAAATGATACTAAAGGTTCTTTGAGAACAGAATTACTAGCAGCTAAAGCAAACA
AGCACAACCTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTGAAAGCCGCACTGCACCAGA
CAGAAGCCTTAGCAGAGCAAGCCGCAGCCAGAGTGACAGCACTGGTGGCTAAAAAAGCTCATTTGCAATAT
CTAAGGGACTTTAAATTTGAATCCTAACCGCCTTCAAGTGATACGTGAGCGCATTTGATAATAC TAAGCAAGA
TTTGGCTAAAACCTCATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCTAAACAAAGCA
GTCTAGAAGCTACTATTGCTACCACAGAACCAGTTGACTTTGCTTAAAACCTTAGCTAACGAAAAGGAA

SEQUENCE LISTING

TATCGCCACTTAGACGAAGATATAGCTACTGTGCCTGATTTGCAAGTAGCTCCACCTCTTACGGGCGTAAA
ACCGCTATCATATAGTAAGATAGATACTACTCCGCTTGTTC AAGAAATGGTTAAAGAAACGAAACAACATAT
TAGAAGCTTCAGCAAGATTAGCTGCTGAAAATACAAGTCTTGTAGCAGAAGCGCTTGTGGCCAAACCTCT
GAAATGGTAGCAAGTAATGCCATTTGTGTCTAAAATCACATCTTCGATTACTCAGCCCTCATCTAAGACATC
TTATGGCTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTGATGAAAGTACTCAACGTGGGCCC
CACTCGAGCACCACCACCACCAC

SEQ ID NO: 30 amino acid sequence comprising HIS-40a CH

M A S S V G V S H Q V K A D D R A S G E T K A S N T H D D S L P K P E T
I Q E A K A T I D A V E K T L S Q Q K A E L T E L A T A L T K T T A E I
N H L K E Q Q D N E Q K A L T S A Q E I Y T N T L A S S E E T L L A Q G
A E H Q R E L T A T E T E L H N A Q A D Q H S K E T A L S E Q K A S I S
A E T T R A Q D L V E Q V K T S E Q N I A K L N A M I S N P D A I T K A
A Q T A N D N T K A L S S E L E K A K A D L E N Q K A K V K K Q L T E E
L A A Q K A A L A E K E A E L S R L K S S A P S T Q D S I V G N N T M K
A P Q G Y P L E L K K L E A S G Y I G S A S Y N N Y Y K E H A D Q I I
A K A S P G N Q L N Q Y Q D I P A D R N R F V D P D N L T P E V Q N E L
A Q F A A H M I N S V R R Q L G L P P V T V T A G S Q E F A R L L S T S
Y K K T H G N T R P S V Y G Q P G V S G H Y G V G P H D K T I I E D S
A G A S G L I R N D D N M Y E N I G A F N D V H T V N G I K R G I Y D S
I K Y M L F T D H L H G N T Y G H A I N F L R V D K H N P N A P V Y L G
F S T S N V G S L N E H F V M F P E S N I A N H Q R F N K T P I K A V G
S T K D Y A Q R V G T V S D T I A A I K G K V S S L E N R L S A I H Q E
A D I M A A Q A K V S Q L Q G K L A S T L K Q S D S L N L Q V R Q L N D
T K G S L R T E L L A A K A K Q A Q L E A T R D Q S L A K L A S L K A A
L H Q T E A L A E Q A A A R V T A L V A K K A H L Q Y L R D F K L N P N
R L Q V I R E R I D N T K Q D L A K T T S S L L N A Q E A L A A L Q A K
Q S S L E A T I A T T E H Q L T L L K T L A N E K E Y R H L D E D I A T
V P D L Q V A P P L T G V K P L S Y S K I D T T P L V Q E M V K E T K Q
L L E A S A R L A A E N T S L V A E A L V G Q T S E M V A S N A I V S K
I T S S I T Q P S S K T S Y G S G S S T T S N L I S D V D E S T Q R A A
A L E H H H H H H

SEQ ID NO: 31 polynucleotide sequence comprising HIS-40a-RR NH

ATGGGATCGCATCACCATCACCATCACCGCTAGTAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAG
AGCCTCAGGAGAAACGAAGGCGAGTAATACTCACGACGATAGTTTACCAAACCAGAAACAATTCAAGAGG
CAAAGGCAACTATTTGATGCAGTTGAAAAACTCTCAGTCAACAAAAGCAGAAGTACAGAGCTTGCTACC
GCTCTGACAAAAACTACTGCTGAAATCAACCCTTAAAAGAGCAGCAAGATAATGAACAAAAGCTTTAAC
CTCTGCACAAGAAATTTACACTAATACTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAAC
ATCAAAGAGAGTTAACAGCTACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACT
GCATTTGTCAGAACAAAAGCTAGCATTTTCAGCAGAAACTACTCGAGCTCAAGATTTAGTGGAACAAGTCAA
AACGCTGTAACAAAATATTGCTAAGCTCAATGCTATGATTAGCAATCCTGATGCTATCACTAAAGCAGCTC
AAACGGCTAATGATAATACAAAAGCATTAAGCTCAGAATTTGGAGAAGGCTAAAGCTGACTTAGAAAAATCAA
AAAGCTAAAGTTAAAAAGCAATTGACTGAAGAGTTGGCAGCTCAGAAAGCTGCTCTAGCAGAAAAAGAGGC
AGAAGTTAGTCGCTTTAAATCCTCAGCTCCGTCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAG
CACCGCAAGGCTATCCTCTTGAAGAACTTAAAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTAC
AATAATTATTACAAAGAGCATGCAGATCAAATTATTGCCAAAGCTAGTCCAGGTAATCAATTAATCAATA
CCAAGATATTCCAGCAGATCGTAATCGCTTTGTTGATCCCATAATTTGACACCAGAAGTGCAAAATGAGC
TAGCGCAGTTTGCAGCTCACATGATTAATAGTGTACCGTcGtCAATTAGGTCTACCACCAGTTACTGTTACA
GCAGGATCACAAGAATTTGCAAGATTACTTAGTACCAGCTATAAGAAAACCTCATGGTAATACAAGACCATC
ATTTGCTCAGGACAGCCAGGGGTATCAGGGCATTTATGGTGTGGGCTCATGATAAAAACCTATTATTGAAG
ACTCTCCAGGAGCGTCAGGGCTATTTCGAAATGATGATAACATGTACGAGAATATCGGTGCTTTTAAACGAT
GTGCATACGTGTAATGGTATTAAACGTGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTT
ACACGGAAATACATACGGCCATGCTATTAACCTTTTACGTGTAGATAAACATAACCCTAATGCGCTGTTT
ACCTTGGATTTTCAACCAGCAATGTAGGATCTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACATT
GCTAACCATCAACGCTTTAATAAGACCCCTATAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGT

SEQUENCE LISTING

AGGCACTGTATCTGATACTATTGCAGCGATCAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTGTCCGGCTA
 TTCATCAAGAAGCTGATATTATGGCAGCCCAAGCTAAAAGTAAGTCAACTTCAAGGTAATTAGCAAGCACA
 CTTAAGCAGTCAGACAGCTTAAATCTCCAAGTGAGACAAATTAATGATACTAAAAGGTTCTTTGAGAACAGA
 ATTACTAGCAGCTAAAGCAAAACAAGCACAACCTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCAT
 CGTTGAAAGCCGCACCTGCACCAGACAGAAGCCTTAGCAGAGCAAGCCGCAGCCAGAGTGACAGCACTGGTG
 GCTAAAAAAGCTCAATTTGCAATATCTAAGGGACTTTAAATTTGAATCCTAACCCGCTTCAAGTGATACGTGA
 GCGCATTTGATAATACTAAGCAAGATTTGGCTAAAACCTACCTCATCTTTGTTAAATGCACAAGAAGCTTTAG
 CAGCCTTACAAGCTAAAACAAAGCAGTCTAGAAGCTACTATTGCTACCACAGAACACCAGTTGACTTTGCTT
 AAAACCTTAGCTAACGAAAAGGAATATCGCCACTTAGACGAAGATATAGCTACTGTGCCTGATTTGCAAGT
 AGCTCCACCTCTTACGGGCGTAAAACCGCTATCATATAGTAAGATAGATACTACTCCGCTTGTTCAGAAA
 TGGTTAAAGAAACGAAACAACCTATTAGAAGCTTCAGCAAGATTAGCTGCTGAAAATACAAGTCTTGTAGCA
 GAAGCGCTTGTGGCCAAACCTCTGAAATGGTAGCAAGTAATGCCATTGTGTCTAAAATCACATCTTCGAT
 TACTCAGCCCTCATCTAAGACATCTTATGGCTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTG
 ATGAAAGTACTCAAGT

SEQ ID NO: 32 amino acid sequence comprising HIS-40a-RR NH

M G S H H H H H A S S V G V S H Q V K A D D R A S G E T K A S N T H D
 D S L P K P E T I Q E A K A T I D A V E K T L S Q Q K A E L T E L A T A
 L T K T T A E I N H L K E Q Q D N E Q K A L T S A Q E I Y T N T L A S S
 E E T L L A Q G A E H Q R E L T A T E T E L H N A Q A D Q H S K E T A L
 S E Q K A S I S A E T T R A Q D L V E Q V K T S E Q N I A K L N A M I S
 N P D A I T K A A Q T A N D N T K A L S S E L E K A K A D L E N Q K A K
 V K K Q L T E E L A A Q K A A L A E K E A E L S R L K S S A P S T Q D S
 I V G N N T M K A P Q G Y P L E E L K K L E A S G Y I G S A S Y N N Y Y
 K E H A D Q I I A K A S P G N Q L N Q Y Q D I P A D R N R F V D P D N L
 T P E V Q N E L A Q F A A H M I N S V R R Q L G L P P V T V T A G S Q E
 F A R L L S T S Y K K T H G N T R P S F V Y G Q P G V S G H Y G V G P H
 D K T I I E D S A G A S G L I R N D D N M Y E N I G A F N D V H T V N G
 I K R G I Y D S I K Y M L F T D H L H G N T Y G H A I N F L R V D K H N
 P N A P V Y L G F S T S N V G S L N E H F V M F P E S N I A N H Q R F N
 K T P I K A V G S T K D Y A Q R V G T V S D T I A A I K G K V S S L E N
 R L S A I H Q E A D I M A A Q A K V S Q L Q G K L A S T L K Q S D S L N
 L Q V R Q L N D T K G S L R T E L L A A K A K Q A Q L E A T R D Q S L A
 K L A S L K A A L H Q T E A L A E Q A A A R V T A L V A K K A H L Q Y L
 R D F K L N P N R L Q V I R E R I D N T K Q D L A K T T S S L L N A Q E
 A L A A L Q A K Q S S L E A T I A T T E H Q L T L L K T L A N E K E Y R
 H L D E D I A T V P D L Q V A P P L T G V K P L S Y S K I D T T P L V Q
 E M V K E T K Q L L E A S A R L A A E N T S L V A E A L V G Q T S E M V
 A S N A I V S K I T S S I T Q P S S K T S Y G S G S S T T S N L I S D V
 D E S T Q R

SEQ ID NO: 33 polynucleotide sequence comprising 40N-HIS

ATGCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGGCGAGTAATACTCACGACGATAGTTTACC
 AAAACCAGAAAACAATTCAAGAGGCAAAGGCAACTATTGATGCAGTTGAAAAAATCTCAGTCAACAAAAAG
 CAGAACTGACAGAGCTTGCCTACCGCTCTGACAAAACTACTGCTGAAATCAACCACTTAAAAGAGCAGCAA
 GATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAATTTACACTAATACTCTTGCAAGTAGTGAGGAGAC
 GCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCTACTGAAACAGAGCTTCATAATGCTCAAG
 CAGATCAACATTCAAAAGAGACTGCATTGTGAGAACAAAAAGCTAGCATTTTCAGCAGAACTACTCGAGCT
 CAAGATTTAGTGGAAACAAGTCAAACGCTCTGAACAAAAATATTGCTAAGCTCAATGCTATGATTAGCAATCC
 TGATGCTATCACTAAAGCAGCTCAAACGGCTAATGATAATACAAAAGCATTAAGCTCAGAATTGGAGAAGG
 CTAAGCTGACTTAGAAAAATCAAAGCTAAAGTTAAAAGCAATTGACTGAAGAGTTGGCAGCTCAGAAA
 GCTGCTCTAGCAGAAAAAGAGGAGCAACTTAGTCGTCTTAAATCCTCAGCTCCGCTCAAGATAGCAT
 TGTGGGTAATAATACCATGAAAGCACCAGGCTATCCTCTTGAAGAATTAAAAAATTAGAAGCTAGTG
 GTTATATTGGATCAGCTAGTTACAATAATTATTACAAAGAGCATGCAGATCAAATATTGCCAAAGCTAGT
 CCAGGTAATCAATTAATCAATACCAAGCGGCCGCACTCGAGCACCACCACCACCACCACC

SEQUENCE LISTING

SEQ ID NO: 34 amino acid sequence comprising 40N-HIS

M Q V K A D D R A S G E T K A S N T H D D S L P K P E T I Q E A K A T I
 D A V E K T L S Q Q K A E L T E L A T A L T K T T A E I N H L K E Q Q D
 N E Q K A L T S A Q E I Y T N T L A S S E E T L L A Q G A E H Q R E L T
 A T E T E L H N A Q A D Q H S K E T A L S E Q K A S I S A E T T R A Q D
 L V E Q V K T S E Q N I A K L N A M I S N P D A I T K A A Q T A N D N T
 K A L S S E L E K A K A D L E N Q K A K V K K Q L T E E L A A Q K A A L
 A E K E A E L S R L K S S A P S T Q D S I V G N N T M K A P Q G Y P L E
 E L K K L E A S G Y I G S A S Y N N Y Y K E H A D Q I I A K A S P G N Q
 L N Q Y Q A A A L E H H H H H H H

SEQ ID NO: 35 amino acid sequence comprising GAS 117

MTLKKHYLLSLLALVTVGAAFNNTSQSVSAQVYSNEGYHQHLTDEKSHLQYSKDNAQLQLRNILDGYQNDLGRHYSSYYYYNLRTVMGLSSEQDIEKHYEELKNKLHDMYNHY

SEQ ID NO: 36 polynucleotide sequence encoding GAS 117

ATGACACTAAAAAACACTATTATCTTCTCAGCCTGCTAGCTCTTGTAACGGTTGGTGCTGCCTTTAACAC
 AAGCCAGAGTGTCTAGTGCACAAGTTTATAGCAATGAAGGGTATCACCCAGCATTGACTGATGAAAAATCAC
 ACCTGCAATATAGTAAAGACAACGCACAACCTCAATTGAGAAATATCCTTGACGGCTACCAAATGACCTA
 GGGAGACACTACTCTAGCTATTATTACTACAACCTAAGAACCCTTATGGGACTATCAAGTGAGCAAGACAT
 TGAAAAACACTATGAAGAGCTTAAGAACAAGTTACATGATATGTACAATCATTATTAA

SEQ ID NO: 37 amino acid sequence comprising GAS 117 leader sequence

TLKKHYLLSLLALVTVGA

SEQ ID NO: 38 amino acid sequence comprising fragment of GAS 117 where leader sequence is removed

AFNNTSQSVSAQVYSNEGYHQHLTDEKSHLQYSKDNAQLQLRNILDGYQNDLGRHYSSYYYYNLRTVMGLSS
 EQDIEKHYEELKNKLHDMYNHY

SEQ ID NO: 39 amino acid sequence comprising GAS 130

MSHMKKRPEVLSPAGTLEKLVKVAIDYGADAVFVGGQAYGLRSRAGNFSMEELQEGIDYAHARGAKVYVAAN
 MVTHEGNEIGAGEWFRQLRDMGLDAVIVSDPALIVICSTEAPGLEIHLSTQASSTNYETFEFWKAMGLTRV
 VLAREVNMAELAEIRKRTDVEIEAFVHGAMCISYSGRCVLSNHMSHRDANRGGCSQSCRWKYDLYDMPFGG
 ERRSLKGEIPEYSSMSVDMCMIDHIPDLIENGVDLSKIEGRMKS IHVSTVTNICYKAAVGAYMESPEAFY
 AIKEELIDELWKVAQRELATGFYYGIPTENEQLFGARRKIPQYKVFVGEVFAFDSASMTATIRQNRVIMEGD
 RIECYGPGFRHFETVVKDLHDADGQKIDRAPNPMELLTISLPREVKPGDMIRACKEGLVNLVYQKDGTSKTV
 RT

SEQ ID NO: 40 polynucleotide sequence encoding GAS 130

ATGTCACATATGAAAAACGTCCTCCGAGGCTTATCACCTGCTGGAACACTTGAAAAATTTAAAAGTTGCGAT
 TGACTATGGCGCAGATGCTGTTTTTGTGGAGGGCAGGCCTATGGCCTAAGAAGCCGCGCTGGTAACTTCT
 CTATGGAAGAATTGCAAGAAGGCATTGATTATGCACATGCGCGTGGAGCTAAGGCTATGTTGCTGCTAAC
 ATGGTTACCCACGAAGGGAACGAAATTGGTGCAGGGCAGTGGTTTTCGTCAACTGCGTGATATGGGGCTTGA
 TGCGGTCATTGTTTCAGATCCAGCCTTGATTGTTATTTGTTCAACAGAAGCCCCAGGTTTGGAAATTCATT
 TGTCAACGCAAGCTTCATCTACCAATTACGAGACCTTTGAATTTTGGAAAGCCATGGGCTTGACCCGAGTT
 GTTTTAGCTCGCGAGGTTAATATGGCCGAGTTAGCAGAAATCCGCAAGCGGACAGATGTGGAAATTTGAAGC
 ATGCCAACAGGGGCGGCTGCTCACAGTCTTGCCGCTGGAAGTATGATTTGTATGACATGCCATTTGGAGGA
 GAGCGCGCTCCTTAAAAAGGGGAAATTCAGAAGACTATTCTATGTCTCTGTTGACATGTGTATGATTGA
 CCATATTCCTGACCTGATTGAAAAATGGGGTTGATAGCTTAAAAATTTGAAGGCCGAATGAAATCTATCCACT
 ACGTCTCAACCGTAACCAACTGTTACAAGCGGCTGTAGGTGCTTACATGGAAAGCCCAGAAGCTTTTTTAT
 GCTATCAAAGAGGAATTGATTGACGAGTTGTGGAAGGTTGCCAGCGGAGTTGGCTACAGGTTTTTACTA
 TGGTATCCCAACTGAAAAATGAACAATTTATTTGGTGTCTCGCCGCAAAATTTCCACAATATAAATTTGTCGGAG
 AAGTAGTTGCCTTTGACTCAGCTAGCATGACAGCGACCATTCGTCAGCGTAATGTCATCATGGAAGGCGAT

SEQUENCE LISTING

CGGATTGAATGTTATGGACCAGGTTTCCGTCATTTTGAAACGGTTGTTAAGGACTTACATGATGCGGATGG
 CCAAAGATTGACCGTGCCCAAATCCAATGGAACCTTAACCATCTCTTTACCGAGAGAAGTTAAGCCAG
 GGGATATGATTAGGGCTTGCAAGGAAGGTCTGGTTAACCTCTATCAAAAAGATGGCACCAGTAAAACCTGTT
 AGAACATAG

SEQ ID NO: 41 amino acid sequence comprising GAS 277

MTTMQKTISLLSLALLIGLLGTSGKAISVYAQDQHTDNVIAESTISQVSVEASMRGTEPYIDATVTTDQPV
 RQPTQATITLKDASDNTINSWVYTMAAQRRFTAWFDLTGQKSGDYHVTVTVHTQEKAVTGQSGTVHFDQN
 KARKTPTNMQQKDTSKAMTNSVDVDTKAQTNQSANQEIDSTSNPFRSATNHRSTSLKRSTKNEKLTPTASN
 SQKNGSNKTKMLVDKKEVKPTSKRGFPWVLLGLVVSLAAGLFIAIQVSRRK

SEQ ID NO: 42 polynucleotide sequence encoding GAS 277

ATGACAACATATGCAAAAAACAATTAGCTTATTATCACTAGCTTTACTTATTGGTTTGC³TGGGGACTTCTGG
 CAAAGCCATATCTGTGTATGCACAAGATCAGCACACTGATAATGTTATAGCTGAATCAACTATTAGTCAGG
 TCAGTGTGGAAGCCAGTATGCGTGGAACAGAACCTTATATTGATGCTACAGTCACCACAGATCAACCTGTC
 AGACAACCAACTCAGGCAACGATAACACTTAAAGACGCTAGTGATAATACTATTAATAGTTGGGTATATAC
 TATGGCAGCGCAACAGCGTCGTTTTACAGCTTGGTTTGATTTAACTGGACAAAAGAGTGGTGACTATCATG
 TAACTGTCACCGTTCATACCTCAAGAAAAGGCAGTAACTGGTCAATCAGGAACTGTTCAATTTTGATCAAAAC
 AAAGCTAGAAAAACACCAACTAATATGCAACAAAAGGATACTTCTAAAAGCAATGACGAATTCAGTCGATGT
 AGACACAAAAGCTCAAACAAATCAATCAGCTAACCAAGAAATAGATTCTACTTCAAATCCTTTT³CAGATCAG
 CTACTAATCATCGATCAACTTCCTTAAAGCGATCTACTAAAAATGAGAACTTACACCAACTGCTAGTAAT
 AGCCAAAAAACCGTAGCAACAAGACAAAAATGCTAGTGGACAAAAGAGGAAGTAAAACCTACTTCAAAAAG
 AGGATTCCCTTGGGTCTTATTAGGTCTAGTAGTCAGTTTAGCTGCAGGTTATTTTATAGCTATTCAAAAAG
 TATCTAGACGAAAATAA

SEQ ID NO: 43 amino acid sequence comprising N-terminal leader sequence of GAS 277

TTMQKTI³SLLSLALLI³GLLGTSGKAI³SVYA

SEQ ID NO: 44 amino acid sequence comprising fragment of GAS 277 where N-terminal leader sequence is removed

QDQHTDNVIAESTISQVSVEASMRGTEPYIDATVTTDQPV³RQPTQATITLKDASDNTINSWVYTMAAQRR
 FTAWFDLTGQKSGDYHVTVTVHTQEKAVTGQSGTVHFDQNKARKTPTNMQQKDTSKAMTNSVDVDTKAQTN
 QSANQEIDSTSNPFRSATNHRSTSLKRSTKNEKLTPTASNSQKNGSNKTKMLVDKKEVKPTSKRGFPWVLL
 GLVVS³LAAGLFIAIQVSR

SEQ ID NO: 45 amino acid sequence comprising GAS 236

MTQMNYTGKVKRVAIIANGKYQSKRVASKLFSVFKDDPDFYLSKKNPDIVISIGGDGMLLSAFHMYEKELD
 KVRVFGIHTGHLGFYTDYRDFEVDKLI³DNLRKDKGEQISYPILKVAITLDDGRVVKARALNEATVKRIEKT
 MVADVIIINHVKFESFRGDGISVSTPTGSTAYNKS³LGGAVLHPTIEALQLTEISSLN³RVFRTL³LGSSIIIPK
 KDKIELV³PKRLGIY³TISIDNKTYQLKNVTKVEYFIDDEKIH³FVSSPSHT³SFWERV³KDAF³IGEIDS

SEQ ID NO: 46 polynucleotide sequence encoding GAS 236

ATGACACAGATGAATTATACAGGTAAGGTA³AAACGAGTTGCTATTATTGCAAA³TGGTAAGTACCAAAGTAA
 ACGCGTCGCTCCAAACTTTTCTCCGTA³TTTAAAGATGATCCTGATTTCTATCTTTCAAAGAAAATCCGG
 ATATTGTGATTTCTATTGGCGGAGATGGGATGCTCTTATCTGCCTTTACATGTATGAAAAAGAATTAGAT
 AAGGTACGTTTTGTAGGAATCCACACCGGTCATCTTGGCTTTTATACCGATTATAGGGATTTTGAAGTTGA
 TAAATTAATTGATAATTTAAGAAAAGACAAGGGAGAACAATCTCTTATCCGATTTTAAAAGTTGCTATTA
 CTTTAGATGATGGTCGTGTGGTTAAAGCGCTGCTTTGAATGAAGCGACGGTTAAGCGTATTGAAAAAACG
 ATGGTAGCAGATGTTATTA³TAA³CCATGTCAAATTTGAAAGCTTCCGAGGTGATGGGATTT³CAGTATCGAC
 CCCGACAGGGAGCACAGCCTACAATAAATCTTTAGGTGGTGTCTTGCATCCGACGATTGAAGCGCTGC
 AATTGACGGAAATTTCCAGTCTTAATAACCGTGTCTTTAGAACCTTGGGCTCATCAATCATTATTTCCAAA
 AAAGATAAGATTGAGTTAGTGCCAAAACGATTAGGAATTTATACCATTTCCATGATAATAAAACCTATCA
 GTTAAAAAATGTGACGAAGGTGGAGTATTTTATCGACGATGAGAAAATTCATTTTGTTCCTCTCCGAGTC
 ATACGAGCTTTTGGGAAAGGGTCAAGGATGCCTTTATTGGAGAGATTGACTCATGA

SEQUENCE LISTING

SEQ ID NO: 47 amino acid sequence comprising N-terminus leader sequence of GAS 236
MTQM

SEQ ID NO: 48 amino acid sequence comprising a fragment of GAS 236 where the N-terminal leader sequence is removed

NYTGKVKRVAI IANGKYQSKRVASKLFSVFKDDPDFYLSKKNPDIVISIGGDGMLLSAFHMYEKELDKVRF
VGIHTGHLGFFYTDYRDFEVDKLI DNLRKDKGEQISYPILKVAITLDDGRVVKARALNEATVKRIEKTMOVAD
VIINHVKFESFRGDGIVSSTPTGSTAYNKSLGGAVLHPTIEALQLTEISSLNNRVFRTLGSIIIPKKDKI
ELVPKRLGIYITISIDNKTYQLKNVTKVEYFIDDEKIHVSVSSPHTSFWERVKDAFIGEIDS

SEQ ID NO: 49 amino acid sequence comprising GAS 389

MRNEMAKIMNVTGEEVIALAATYMTKADVAFVAKALAYATAAHFYQVRKSGEPYIVHPIQVAGILADLHLD
AVTVACGFLHDVVEDTDITLDEIEADFGHDARDIVDGVTKLGEVEYKSHEEQLAENHRKMLMAMSKDIRVI
LVKLADRLHNMRTLKHLRDKQERI SRETMEIYAPLAHRLGISRIKWELEDLAFRYLNETEFYKISHMMKE
KRREREALVEAIVSKVKTYYTQQGLFGDVYGRPKHIYSIYRKMMDKKRFDQIFDLIAIRCVMETQSDVYA
MVGYIHELWRPMPGRFKDYIAAPKANGYQSIHTTVYGPKGPIEIQIRTKDMHQVAEYGVAAHWAYKKGVRG
KVNQAEQAVGMNWKELVELQDASNGDAVDFVDSVKEDIFSERIYVFTPTGAVQELPKESGPIDFAYAIHT
QIGEKATGAKVNGRMVPLTAKLKTGDVVEIITNANSFGPSRDWVKLVKTNKARNKIRQFFKNQDKELSVNK
GRDLLVSYFQEQGYVANKYLDKKRIEAILPKVSVKSEESLYAAVGFGDISPISVFNKLEKERREEERAKA
KAEAEELVKGGEVKHENKDVLVKVRSENGVIOGASGLLMRIAKCCNPVPGDPIDGYITKGRGIAIHRSDCH
NIKSDQGYQERLIEVEWDLNNSKDYQAEIDIYGLNRSGLLNDVLQILSNSTKSI STVNAQPTKDMKFANI
HVSFGIPNLTHLTTVVEKIKAVPDVYSVKRTNG

SEQ ID NO: 50 polynucleotide sequence encoding GAS 389

ATGAGGAACGAAATGGCAAAAATAATGAACGTAACAGGAGAAGAAGTCATTTGCCTTAGCGGCCACCTATAT
GACCAAGGCTGATGTGGCTTTTGTGGCAAAGGCTTTAGCATATGCAACAGCGGCCATTCTACCAAGTGA
GAAAGTCAGCGCAACCTATATCGTCCATCCGATTTCAGGTGGCGGGGATTCGGCTGATTGCATCTGGAT
GCTGTGACAGTTGCTTGTGGCTTTTACATGATGTCTGTAAGAATACGGATATTACCTTAGATGAGATCGA
AGCAGACTTTGGCCATGATGCTCGTGATATCGTTGATGGTGTACCAAGTTAGGTGAAGTTGAGTACAAAT
CTCATGAGGAGCAACTCGCCGAAAACCATCGCAAAATGCTGATGGCTATGTCCAAAGATATTCGCGTGATT
TTGGTGAAATTTGGCTGACCGCTGCATAATATGCGCACCCCTCAAACATTTGCGCAAGGACAAACAAGAGCG
CATTTTCGCGGAAACCATGGAAATCTATGCCCCCTTGGCGCATCGTTTGGGGATTAGTCGCATCAAATGGG
AACTAGAAGATTTGGCTTTTTCGTTACCTCAATGAAACCGAATTTTACAAAATTTCCCATATGATGAAAGAA
AAACGTCGCGAGCGTGAAGCTTTGGTAGAGGCTATTGTGAGTAAGGTCAAAACCTATACGACACAACAAGG
GTTGTTTGGAGATGTGTATGGCCGACCAAAACACATTTATTCGATTTATCGGAAAATGCGGGCAAAAAGA
AACGATTCGATCAGATTTTGTATCTGATTGCCATTCGTTGTGTGTCATGGAAACGCAAGCGATGTCATAGCT
ATGGTTGGCTATATTCATGAGCTTTGGCGTCCCATGCCAGGCCGCTTCAAGGATTTATATGTCAGCTCCTAA
AGCTAATGGCTACCAGTCTATTCATACCACCGTGTATGGGCCAAAAGGACCTATTGAGATTCAAATCAGAA
CTAAGGACATGCATCAAGTGGCTGAGTACGGGGTGTGCTCACTGGGCTTATAAAAAAGGCGTGCCTGGT
AAGGTCAATCAAGCTGAGCAAGCCGTTGGCATGAACTGGATCAAAGAGCTGGTAGAATTGCAAGATGCCTC
AAATGGCGATGCAGTGGACTTTGTGGATTCGGTCAAAGAAGACATTTTCTGTAACGGATTTATGTCTTTA
CACCGACAGGGGCCGTTTACGAGTTACCAAAAAGAAATCAGGTCCATTTGATTTTGTCTTATGCGATCCATACG
CAAATCGGTGAAAAGCAACAGGTGCCAAAGTCAATGGACGTATGGTTCCCTCTCACTGCCAAGTTAAAAAC
AGGAGATGTGGTTGAAATCATCACC AATGCCAATTCCTTTGGCCCTAGTCGAGACTGGGTA AAACTGGTCA
AAACCAATAAGGCTCGCAACAAAATTCGTGAGTTCTTTAAAAAATCAAGACAAGGAATTTGTCAGTGAATAAA
GGCCGTGATTTGTTGGTGTCTTATTTTCAAGAGCAGGGCTACGTTGCCAATAAATACCTTGACAAAAAACG
CATTGAAGCCATCCTTCCAAAAGTCAAGTGTGAAGAGCGAAGAATCACTCTATGCAGCCGTTGGGTTTGGTG
ACATTAGTCTATCAGTGTCTTTAACAAGTTAACCGAAAAAGAGCGCCGTGAAGAAGAAAGGGCCAAGGCT
AAAGCAGAAGCTGAAGAATTTGGTTAAGGGCGGTGAGGTCAAACACGAAAACAAAGATGTGCTCAAGGTTTCG
CAGTGA AAATGGAGTCATTATCCAAGGAGCATCAGGCCTCTTGATGCGGATTGCCAAGTGTGTAATCCTG
TACCTGGTGATCCTATTGACGGCTACATTACCAAAGGGCGTGGCATTGCGGATTCACAGATCGGACTGTCTAT
AACATTAAAGAGTCAAGATGGCTACCAAGAACGCTTGATTGAGGTCGAGTGGGATTTGGACAATTCGAGTAA
AGATTTACAGGCTGAAATGATATCTATGGGCTCAATCTGAGTGGTCTGCTTAATGATGTGCTCCAAATTT
TATCAAAC TCAACCAAGACATATCGACAGTCAATGCTCAGCCGACCAAGGACATGAAGTTTGTCTAATATT
CACGTGAGCTTTGGCATTCCAAATCTGACGCATCTGACCACTGTTGTGCGAAAAAATCAAGGCAGTTCCAGA
TGTTTATAGCGTGAAGCGGACCAATGGCTAA

SEQUENCE LISTING

SEQ ID NO: 51 amino acid sequence comprising GAS 504

MKTRITELLNIDYPIFQGGMAWVADGDLGAVSNAGGLGIIGGNAPKEVVKANIDRVKAITDRPFGVNM
 LLSPFADDIVDLVIEEGVKVVTGAGNPGKYMERLHQAGIIVVPVPSVALAKRMEKLGVDVIAEGMEAG
 GHIGKLTMSLVRQVVEAVSIPVIAAGGIADGHGAAAAFMLGAEAVQIGTRFVVAKESNAHQNFKDKILAA
 KDIDTVISAQVVGHPVRSIKNKLTSAYAKAEKAFLIGQKTATDIEEMGAGSLRHAVIEGDVVNGSVMAGQI
 AGLVRKEESCEITLKDIIYGAARVIQNEAKRWQSVSIEK

SEQ ID NO: 52 polynucleotide sequence encoding GAS 504

ATGAAAACACGTATTACAGAATTACTTAATATTGATTACCCCATTTTCAAGGAGGAATGGCTTGGGTTGC
 TGATGGTGATTTAGCAGGTGCAGTTTCTAATGCTGGTGGTTTAGGCATTATAGGTGGTGGCAATGCTCCCA
 AAGAAGTCGTTAAAGCTAATATTGATCGTGTCAAAGCTATTACTGATAGACCTTTTGGGGTTAATATCATG
 CTTTTATCTCCTTTTGTGTGATGATATCGTTGATCTGGTCATTGAAGAAGGTGTTAAAGTAGTAACAACAGG
 CGCAGGAAATCCAGGAAAGTATATGGAAAGACTGCACCAGGCGGGTATAATCGTTGTTCCCTGTTGTCCCAA
 GCGTTGCGCTAGCCAAACGTATGGAAAAGCTTGGGGTAGATGCTGTTATGCTGAGGGTATGGAAGCTGGA
 GGACATATTGGCAAGTTAACGACTATGCTTTAGTAAGACAAGTTGTTGAAGCGGTTTCGATTCTCTGTCAT
 TGCGGCAGGTGGTATAGCTGATGGTCATGGTGCAGCAGCAGCATTATGTTAGGAGCAGAGGCTGTTCAA
 TTGGAACCTCGCTTTGTTGTTGCTAAAGAATCCAATGCTCACCAAAATTTTAAAGATAAAAATCTTAGCAGCA
 AAAGATATTGATACGGTGATTTCTGCGCAGGTTGTGGCCACCCTGTCCGTTCTATTAATAAATAAATTGAC
 CTCAGCTTACGCTAAAGCAGAAAAAGCATTTTTAATTGGTCAAAAAACAGCTACTGATATTGAAGAAATGG
 GAGCAGGATCGCTTCGACACGCTGTTATTGAAGGCATGATGATCAATGGATCTGTTATGGCTGGCCAAAT
 GCAGGGCTTGTGAGAAAAGAAGAAAGCTGTGAAACGATTTTAAAAGATATTTATTATGGTGCAGCTCGTGT
 TATTCAAATGAAGCTAAGCGCTGGCAATCTGTTTCAATAGAAAAGTAG

SEQ ID NO: 53 amino acid sequence comprising GAS 509

MTKIYKTTITELVGQTPIIKLNRLIPNEAADVYVKLEAFNPGSSVKDRIALSMIEAAEAEGLISPGDVIIIE
 P TSGNTGIGLAWVGAAGKYRVIIVMPETMSLERRQIIQAYGAELVLTPGAEGMKGAIKAETLAIELGAW
 MPMQFNPNPANSIHEKTTAQEILEAFKEISLDAFVSGVGTGGTLSGVSHVLKKNPETVIYAVEAESAV
 LSGQEPGPHKIQGISAGFIPNTLDTKAYDQIIRVKS KDALETARLTGAKEGFLVGLISSGAALYAAIEVAK
QLGKGGKHLVLTILPDNGERYLSTELYDVPVIKTK

SEQ ID NO: 54 polynucleotide sequence encoding GAS 509

ATGACTAAAATTTACAAAACATAACAGAATTAGTAGGTCAAACACCTATTATCAAACCTTAACCGTTTAA
 TTCCAAACGAAGCTGCTGACGTTTATGTAAAATTAGAAGCTTTTAAACCCAGGATCTTCTGTAAAGATCG
 TATTGCTTTATCGATGATTGAAGCTGCTGAAGCTGAAGGCTGATAAGTCCCTGGTGACGTTATTATCGAA
 CCAACAAGTGTTAATACAGGTATTGGTCTTGGCATGGGTAGGTGCTGCTAAAGGGTATCGAGTCATTATTG
 TTATGCCCGAAACTATGAGCTTGGAAAGACGGCAAAATCATTACAGGCTTATGGTGCAGAGCTTGTCTTAAC
 ACCTGGAGCAGAAGGTATGAAAGGGGCTATTGCAAAAGCTGAAACTTTAGCAATAGAACTAGGTGCTTGG
 ATGCCATATGCAATTTAATAACCCTGCCAATCCAAGCATCCATGAAAAACAACAGCTCAAGAAATTTTGG
 AAGCTTTTAAGGAGATTTCTTTAGATGCATTCGTATCTGGTGTGGTACTGGAGGAACACTTTCTGGTGT
 TTCACATGCTTTGAAAAAGCTAACCCTGAAACTGTTATCTATGCTGTTGAAGCTGAAGAAATCTGCTGTC
 TTATCTGGTCAAGAGCCTGGACCACATAAAAATCAAGGTATATCAGCTGGATTTATCCCAAACACGTTAG
 ATACCAAAGCCTATGACCAATATCCGTGTTAAATCGAAAGATGCTTTAGAAACTGCTCGACTAACAGG
 AGCTAAGGAAGCTTCTTGGTTGGGATTTCTTCTGGAGCTGCTTTTACGCCGCTATTGAAGTCGCTAAA
CAGTTAGGAAAAGGCAACATGTGTTAACTATTTACCAGATAATGGCGAACGCTATTTATCGACTGAAC
TCTATGATGTACCAGTAATTAAGACGAAATAA

SEQ ID NO: 55 amino acid sequence comprising C-terminus transmembrane region of GAS 509

FLVGISSGAALYAAIEVAKQLGKGGKHLVLTILPDNGERYLSTELYDVPVIKTK

SEQ ID NO: 56 amino acid sequencing comprising a fragment of GAS 509 where the C-terminal transmembrane region is removed

MTKIYKTTITELVGQTPIIKLNRLIPNEAADVYVKLEAFNPGSSVKDRIALSMIEAAEAEGLISPGDVIIIEP
 TSGNTGIGLAWVGAAGKYRVIIVMPETMSLERRQIIQAYGAELVLTPGAEGMKGAIKAETLAIELGAWMP

SEQUENCE LISTING

MQFNPNANPSIHEKTTAQEILEAFKEISLDAFVSGVGTGGTLSGVSHVLKKNPETVIYAVEAEESAVALSG
QEPGPHKIQGISAGFIPNTLDTKAYDQIIRVKSKDALETARLTGAKEG

SEQ ID NO: 57 amino acid sequence comprising GAS 366

MKVISNFQNKKILILGLAKSGEAAKLLTKLALVTVNDSKPFQNPAAQALLEEGIKVICGSHPVVELLDE
NFEYMVKNPGIPYDNPMVKRALAKEIPILTEVELAYFVSEAPIIGITGSNGKTTTTMIADVLNAGGQSAL
LSGNIGYPASKVVQKAIAGDTLVMELSSFQLVGVNAFRPHIAVITNLMPHTLDYHGSEFEDYVAAKMMIQAQ
MTESDYLLILNANQEISATLAKTTKATVIFPSTQKVVDGAYLKDGLLYFKEQAIIAATDLGVPGSHNIENAL
ATTIAVAKLSGIADDIIAQCLSHFGGVKHRLQRVGQIKDITFYNDSKSTNILATQKALSGFDNSRLILIAGG
LDRGNEFDDLVPDLLGLKQMIILGESAERMKRAANKAEVSYLEARNVAEATELAFKLAQTGDTILLSPANAS
SWDMYPNFEVRGDEFLATFDCLRGDA

SEQ ID NO: 58 polynucleotide sequence encoding GAS 366

ATGAAAGTGATAAGTAATTTTCAAAACAAAAAATATTAATATTGGGGTTAGCCAAATCGGGCGAAGCAGC
AGCAAAATTTATGACCAAACCTGGGTGCTTTAGTGACTGTTAATGATAGTAAACCATTTGACCAAAATCCAG
CGGCACAAGCCTTGTGGAAGAGGGGATTAAGGTCATTTGTGGTAGCCACCCAGTAGAATTATTAGATGAG
AAGTTGAGTACATGGTTAAAAACCCTGGGATTCCTTATGATAATCCTATGGTTAAACCGCCCTTGCAAA
GGAAATTCCTACTTACTGACTGAAGTAGAATTGGCTTATTTCTGATCTGAAGCGCCTATTATCGGGATTACAG
GATCAAACGGGAAGACAACCACAACGACAATGATTGCCGATGTTTTGAATGCTGGCGGGCAATCTGCACTC
TTATCTGGAAACATTTGGTTATCCTGCTTCAAAAGTTGTTCAAAAAGCAATGCTGGTGATACTTTGGTGAT
GGAATTGTCTCTTTTCAATTAGTGGGAGTGAATGCTTTTCGCCCTCATATTGCTGTCATCACTAATTTAA
TGCCGACTCACTGGACTATCATGGCAGTTTTGAGGATTATGTTGCTGCTAAATGGATGATTCAAGCTCAG
ATGACAGAAATCAGACTACCTTATTTTAAATGCTAATCAAGAGATTTTCAGCAACTCTAGCTAAGACCACAA
AGCAACAGTGATTCCTTTTTCAACTCAAAAAGTGGTTGATGGAGCTTATCTGAAGGATGGAATACTCTATT
TTAAAGAACAGGCGATTATAGCTGCAACTGACTTAGGTGTCCCAGGTAGCCACAACATTGAAAATGCCCTA
GCAACTATTGCACTGGCCAAGTTATCTGGTATTGCTGATGATATTTATGCCCAGTGCCTTTACATTTTGG
AGGCGTTAAACATCGTTTTGCAACGGGTTGGTCAAATCAAAGATATTACCTTCTACAATGACAGTAAGTCAA
CCAATATTTTAGCCACTCAAAAAGCTTTATCAGGTTTTGATAACAGTCGCTTGATTTTGATTGCTGGCGGT
CTAGATCGTGCAATGAATTTGACGATTTGGTGCCAGACCTTTTAGGACTTAAGCAGATGATTATTTTGGG
AGAATCCGCAGAGCTATGAAGCGAGCTGCTAACAAGCAGAGGTCCTTATCTTGAAGCTAGAAATGTGG
CAGAAGCAACAGAGCTTGCTTTTAAAGCTGGCCCAACAGGCGATACTATCTTGTCTTAGCCAGCCAATGCT
AGCTGGGATATGTATCCTAATTTGAGGTTTCGTGGGGATGAATTTTGGCAACCTTTGATTGTTAAGAGG
AGATGCCTAA

SEQ ID NO: 59 amino acid sequence comprising N-terminal leader sequence of GAS 366

MKVISNFQNKKILILGLAKSGEAAA

SEQ ID NO: 60 amino acid sequence comprising a fragment of GAS 366 where the N-terminal leader sequence is removed

KLLTKLALVTVNDSKPFQNPAAQALLEEGIKVICGSHPVVELLDENFEYMVKNPGIPYDNPMVKRALAKE
IPILTEVELAYFVSEAPIIGITGSNGKTTTTMIADVLNAGGQSALLSGNIGYPASKVVQKAIAGDTLVMEL
LSSFQLVGVNAFRPHIAVITNLMPHTLDYHGSEFEDYVAAKMMIQAQMTESDYLLILNANQEISATLAKTTKA
TVIFPSTQKVVDGAYLKDGLLYFKEQAIIAATDLGVPGSHNIENALATTIAVAKLSGIADDIIAQCLSHFGG
VKHRLQRVGQIKDITFYNDSKSTNILATQKALSGFDNSRLILIAGGLDRGNEFDDLVPDLLGLKQMIILGE
SAERMKRAANKAEVSYLEARNVAEATELAFKLAQTGDTILLSPANASWDMYPNFEVRGDEFLATFDCLRGDA

SEQ ID NO: 61 amino acid sequence comprising GAS 159

MRKLYSFLAGVLGVIVILTSLSFILQKKSGSGSQSDKLVIVNWGDYIDPALLKKFTKETGIEVQYETFDNS
EAMYTKIKQGGTTYDIAVPSDYITDKMIKENLLNKLDKSKLVGMDNIGKEFLGKSFDPQNDYSLPYFWGTV
GIVYNDQLVDKAPMHWEDLWRPEYKNSIMLIDGAREMLGVGLTTFGYSVNSKNLEQLQAAERKLQQLTPNV
KAIVADEMKGMIQGDAAIGITFSGEASEMLDSNEHLHYIVPSEGSNLWFDNLVLPKTMKHEKEAYAFLN
INRPENAAQNAAAYIGYATPNKKAKALLPDEIKNDPAFYPTDDIIKKLEVDNLGSRWLGVIYNDLYLQFKMY
RK

SEQUENCE LISTING**SEQ ID NO: 62** polynucleotide sequence encoding GAS 159

ATGCGTAAACTTTATTCCTTTCTAGCAGGAGTTTGGGTGTTATTGTTATTTAACAAGTCTTTCTTTTCAT
CTTGCAGAAAAATCGGGTTCTGGTAGTCAATCGGATAAATTAGTTATTTATAACTGGGGAGATTACATTG
ATCCAGCTTTTGCTCAAAAAATTCACCAAAGAAACGGGCATTGAAGTGCAGTATGAACTTTTCGATTCCAAT
GAAGCCATGTACACTAAAATCAAGCAGGGCGGAACCACTTACGACATTGCTGTTCCCTAGTGATTACACCAT
TGATAAAATGATCAAAGAAAACCTACTCAATAAGCTTGATAAGTCAAATTAGTTGGCATGGATAATATCG
GGAAAGAATTTTTAGGGAAAAGCTTTGACCCACAAAACGACTATTCCTTTGCCCTATTTCTGGGGAACCGTT
GGGATTGTTTTATAATGATCAATTAGTTGATAAAGCGCCTATGCACCTGGGAAGATCTGTGGCGTCCAGAATA
TAAAAATAGTATTATGCTGATTGATGGAGCGCGTGAAATGCTAGGGGTTGGTTTAAACAACTTTTGGTTATA
GTGTGAATTCATAAAATCTAGAGCAGTTGCAGGCAGCCGAGAGAAAACGCAGCAGTTGACGCCGAATGTT
AAAGCCATTGTAGCAGATGAGATGAAAGGCTACATGATTC AAGGTGACGCTGCTATTGGAATTACCTTTTC
TGGTGAAGCCAGTGAGATGTTAGATAGTAACGAACACCTTCACTACATCGTGCCCTCAGAAGGGTCTAACC
TTTTGGTTTTGATAATTTGGTACTACCAAAAACCATGAAACACGAAAAAGAAGCTTATGCTTTTTTTGAACTTT
ATCAATCGTCTGAAAATGCTGCGCAAAATGCTGCATATATTGGTTATGCGACACCAAAATAAAAAAGCCAA
GGCCTTACTTCCAGATGAGATAAAAAATGATCCTGCTTTTTATCCAACAGATGACATTATCAAAAAATTGG
AAGTTTATGACAATTTAGGGTCAAGATGGTTGGGGATTTATAATGATTATACCTCCAATTTAAAATGTAT
CGCAATAA

SEQ ID NO: 63 amino acid sequence comprising N-terminal leader sequence of GAS 159

MRKLYSFLAGVLGVIVILTSLSFI

SEQ ID NO: 64 amino acid sequence comprising a fragment of GAS 159 where the N-terminal leader sequence is removed

LQKKS GSGS QSDKLV IYNWGDY IDPALLK KFTKETG IEVQYETFDS NEAMYTKI KQGGTTYD IAVPSDYTI
 DKMIKENLLNKL DKS KLVGMDNIGKEFLGKSFDPQNDYSLPYFWGTVGIVYNDQLVDKAPMHWEDLWRPEY
 KNSIMLIDGAREMLGVGLTTFGYSVNSKNLEQLQAAERKLQQLTPNVKAI VADEMKG YMIQGDA AIGITFS
 GEASEMLDSNEHLHY IVPSEGSNLWFDNLVLPKTMKHEKEAYAF LNF INRPENAAQNAAY IGYATPNKKAK
 ALLPDEIKNDFAFYPTDDI I K KLEVYDNLGSRWLG IYNDLYLQFKMYRK

SEQ ID NO: 65 amino acid sequence comprising C-terminal hydrophobic sequence of GAS 159

WLG IYNDLYLQFKMYRK

SEQ ID NO: 66 amino acid sequence comprising a fragment of GAS 159 where the C-terminal hydrophobic region is removed

MRKLYSFLAGVLGVIVILTSLSFI LQKKS GSGS QSDKLV IYNWGDY IDPALLK KFTKETG IEVQYETFDSN
 EAMYTKI KQGGTTYD IAVPSDYTI DKMIKENLLNKL DKS KLVGMDNIGKEFLGKSFDPQNDYSLPYFWGTV
 GIVYNDQLVDKAPMHWEDLWRPEYKNSIMLIDGAREMLGVGLTTFGYSVNSKNLEQLQAAERKLQQLTPNV
 KAI VADEMKG YMIQGDA AIGITFS GEASEMLDSNEHLHY IVPSEGSNLWFDNLVLPKTMKHEKEAYAF LNF
 INRPENAAQNAAY IGYATPNKKAK ALLPDEIKNDFAFYPTDDI I K KLEVYDNLGSR

SEQ ID NO: 67 amino acid sequence comprising a fragment of GAS 159 where the N-terminal leader sequence and the C-terminal hydrophobic region is removed

LQKKS GSGS QSDKLV IYNWGDY IDPALLK KFTKETG IEVQYETFDS NEAMYTKI KQGGTTYD IAVPSDYTI
 DKMIKENLLNKL DKS KLVGMDNIGKEFLGKSFDPQNDYSLPYFWGTVGIVYNDQLVDKAPMHWEDLWRPEY
 KNSIMLIDGAREMLGVGLTTFGYSVNSKNLEQLQAAERKLQQLTPNVKAI VADEMKG YMIQGDA AIGITFS
 GEASEMLDSNEHLHY IVPSEGSNLWFDNLVLPKTMKHEKEAYAF LNF INRPENAAQNAAY IGYATPNKKAK
 ALLPDEIKNDFAFYPTDDI I K KLEVYDNLGSR

SEQ ID NO: 68 amino acid sequence comprising GAS 217

MAQRIIVITGASGGLAQAI VKQLPKEDSLILLGRNKERLEHCYQHIDNKECLELDITNPVAIEKMVAQIYQ
 RYGRIDVLINNAGYGAFKGFEEFSAQEIADMFOVNTLASIHFACLI GQKMAEQGQHLINIVSMAGLIASA
 KSSIYSATKFALIGFSNALRLELADKGVYVTTVNP GPIATKFFDQADPSGHYLESVGKFTLQPNQVAKRLV
 SII GKNKRELNLPFSLAVTHQFYTLFPKLSDY LARKVFNKY

SEQ ID NO: 69 polynucleotide sequence encoding GAS 217

SEQUENCE LISTING

ATGGCACAAAGAATCATTTGTTATCACGGGAGCTTCTGGAGGACTGGCTCAGGCAATTGTTAAGCAGTTACC
CAAGGAAGACAGCTTGATTTTATTAGGACGTAACAAAGAACGCTAGAACACTGTTATCAGCATATTGACA
ACAAAGAATGCCCTCGAGTTGGATATTACCAATCCAGTAGCCATTGAGAAAATGGTCGCCCAGATTTACCAG
CGCTATGGCCGTATTGATGTC'TTGATTAATAATGCTGGCTACGGAGC'TTCAAAGGCTTTGAAGAGTTTTC
TGCCCAAGAAATAGCTGATATGTTTCAGGTAAACACCCTAGCGAGCATTCACTTTGCTTGGCTTGATTGGTC
AGAAAATGGCAGAGCAGGGGCAAGGTCACCTTATTAATATTTGTGTCCATGGCAGGCTTGATTGCCGTACGC
AAATCGAGCATTATTACGCCACCAAGTTTGCCCTTATCGGATTTTCCAATGCCCTTCGTTAGAAATTAGC
GGATAAAGGGGTTTACGTGACCACCGTGAATCCAGGTCCCATTTGCCACCAAGTTTTTTTGACCAAGCTGACC
CGTCTGGACATTATTGGAAAGCGTTGGTAAATTTACTCTCCAACCAATCAAGTGGCTAAGCGTTTGGTT
TCTATTATCGGGAAAAATAAACGAGAATTGAATTTGCCCTTTAGTTTAGCGGTGACCCATCAATTTTACAC
CCTTTTCCCTAAATTATCTGATTATCTTGCAAGAAAGGTATTTAATTATAAATGA

SEQ ID NO: 70 amino acid sequence comprising GAS 309

MIEKYLESSIESKQLIVLFFKTSYLPITEVAEKTGLTFLQLNHYCEELNAFFPGSLSMITQKRMISCFQFT
HPFKETYLYQLYASSNVLQLLAFLIKNGSHSRPLTDFARSHFLSNSSAYRMREALIPLLRNFELKLSKNKI
VGEEYRIRYLIALLYSKFGIKVYDLTQQDKNTIHSFLSHSSTHLKTS PWLSESFYDILLALSWKRHQFS
VTIPQPRIFQQLKFLVYDSLKSSHDIIETYCQLNFSAGDLDYLYLIYITANNFASLQWTFPEHIRQYQCQ
LFEENDTFRLLLNPIITLLPNLKEQKASLVKALMFFSKSFLFNLQHFIPETNLFVSPYKGNQKLYTSLKL
IVEEWMKLPKGRDLNHHKHFHLFCHYVEQSLRNIQPLVVVVFVASFNAHLLTDSFPRYFSDKSIDPHSY
YLLQDNVYQIPDLKPDLVITHSQLIPFVHHELTGKIAVAEISFDESILSIQELMYQVKEEFQADLTKQLT

SEQ ID NO: 71 polynucleotide sequence encoding GAS 309

TTGATAGAAAAATAC'TTGGAAATCATCAATCGAATCAAAAATGTCAGTTAATTGCTTGT'TTTTAAAGACATC
TTATTTGCCAATAACTGAGGTAGCAGAAAAAACTGGCTTAACCTTTTACAACATAAACCATTATTTGTGAGG
AACTGAATGCC'TTTTCCCTGGTAGTCTGTCTATGACCATCCAAAAAGGATGATATCTTGCCAAATTTACA
CATCTTTTAAAGAAACTTATCTTTACCAACTCTATGCATCATCTAATGTCTTACAATTACTAGCCTTTT
AATAAAAAATGG'TTCCCACTCTCGTCCCTTACGGATTTTGAAGAAGTCATTTTATCAAACCTCCTCAG
CTTATCGGATGCGCGAAGCATTGATTCTTTATTAAGAACTTTGAATAAAACCTCTAAGAACAAGATT
GTCGGTGAGGAATATCGCATCCGTTACCTCATCGCTCTGCTATATAGTAAGTTTGGCATTAAGTTTATGA
CTTGACGCAGCAAGACAAAACACTATTCATAGCTTTTATCCCATAGTTCCACCCACCTTAAAACCTCTC
CTTGGTTATCGGAATCGTTTCTTTCTATGACATTTTATTAGCTTTATCGTGGAAAGCGGCATCAATTTTCG
GTAACTAT'TCCCAAACCAGAATTTTCAACAATTAAAAAAAC'TTTTGTCTACGATTCTTTGAAAAAAG
TAGCCATGATATATCGAAACTTACTGCCAACTAAACTTTTTCAGCAGGAGATTGGACTACCTCTATTTAA
TTTATATCACCAGTAATAATCTTTTTCGAGCTTACAATGGACACCTGAGCATATCAGACAATATTTGCAA
CTTTTGAAGAAATGATACTTTTTCGCTGCTTTTAAATCCTATCATCACTCTTTTACCTAACCTAAAAGA
GCAAAAAGGCTAGTTTAGTAAAAGCTCTTATGTTT'TTCAAATCATCTTGT'TTAATCTGCAACATTTTA
TTCTTGAGACCAACTTATTCGTTTCTCCGTAATAAAGGAAACCAAAACTCTATACGCTCTTAAAGTTA
ATTGTCGAAGAGTGGATGGCCAAACTTCTTGGTAAGCGTGACTTGAACCATAAGCATTTCATCTTTTTCG
CCACTATGTCGAGCAAAGTCTAAGAAATATCCAACCTCCTTTAGTTGTTGTTTTCGTAGCCAGTAATTTTA
TCAATGCTCATCTCCTAACGGATTCTTTTCCAAGGTATTTCTCGGATAAAAAGCATTGATTTTCATTCCTAT
TATCTATTGCAAGATAATGTTTATCAAATTCCTGATTTAAAGCCAGATTGGTGCATCACTCACAGTCAACT
GATTCCTTTTGTTCACCATGAAC'TTACAAAAGGAATGCTGTGTTGAAATATCTTTTATGATGAATCGATT
TGTCTATCCAAGAATTGATGTATCAAGT'PAAAGAGGAAAAATCCAAGCTGATTTAACCAAGCAATTAACA
TAA

SEQ ID NO: 72 amino acid sequence comprising GAS 372

MIQIGKLFAGRYRILKSI GRGGMADVYLANDLILDNEDVAIKVLRNTNYQTDQVAVARFQREARAMELNHP
NIVAIRDIGEEGQQFLVMEYVDGADLKRYIQNHAPLSNNEVVRIMEEVL SAMTLAHQKGI VHRDLKQPNI
LLTKEGVVKTDFGLI AVAFAETSLTQTN SMLG SVHYLSPEQARGSKATI QSDIYAMGIMLFEMLTGHI PYD
GDSAVTIALQH FQKPLPSII EENHNVPQALENVVIRATAKLSDRYGSTFEMSRDLMTALS YNRSRERKII
FENVESTKPLPKVASGPTASVKLS PPTPTVLTQESRLDQTNQTDALQPPTK KKS SGRFLGTLFKILFSFFI
VGVALFVY LILTKPTSVKVPNVAGTSLK VAKQELYDVGLKVGKIRQIESD TVAEGNVVR'DPKAGTAKRQ
SSI LFTVY SIGNKGFDMENYKGLDYQEAMNSLIET YVLPKSKI KIERIVTNEY PENTVI SQSPSAGDKFNP
GKSKITLSVAVSDTITMPMVTEYSYADAVNTLTALGIDASRIKAYVPSSSSATGFVPIHSPSSKAI VSGQS
PYYGTSLSLSDKGEISLYLYPEETHSSSSSSSSSTSSSNSSSINDSTAPGSNTELSPSETTSQTP

SEQUENCE LISTING

SEQ ID NO: 73 polynucleotide sequence encoding GAS 372

ATGATTCAGATTGGCAAATTATTTGCTGGTTCGTTATCGCATTCTGAAATCTATTTGGCCGCGGTGGTATGGC
 GGATGTTTTATTTAGCAAATGACTTGTATCTTGGATAATGAAGACGTTGCAATCAAGGCTTTGCGTACCAATT
 ATCAAACAGATCAGGTAGCAGTTGCGCGTTTCCAACGAGAAGCGCGGGCCATGGCTGAATTTGAACCATCCC
 AATATTTGTTGCCATCCGGGATATAGGTGAAGAAGACGGACAGCAATTTTTAGTAATGGAATATGTGGATGG
 TGCTGACCTAAAGAGATACATTCAAAATCATGCTCCATTATCTAATAATGAAGTGGTTFAGAATTATGGAAG
 AAGTCCTTTTCTGCTATGACTTTAGCCCACAAAAGGAATTGTACACAGAGATTTAAAACCTCAAATATC
 CTACTAACTAAGGAGGGTGTGTCAAAGTAACTGATTTCCGGCATCGCAGTAGCCTTTGCAGAAACAAGCTT
 GACACAACTAATTCGATGTTAGGCAGTGTTCATTACTTGTCTCCAGAACAGGCTCGCGGCTCCAAAGCGA
 CGATTCAAAGTGATATTTATGCGATGGGGATTATGCTCTTTGAGATGTTGACAGGCCATATCCCTTATGAC
 GCGGATAGTGTGTTACGATTGCCTTGCACATTTTCAAAGCCTCTTCCATCTATTTATCGAGGAGAACCA
 CAATGTGCCACAAGCTTTGGAGAATGTTGTTATTTCGAGCAACAGCCAAAGAAATTAAGTGATCGTTACGGGT
 CAACCTTTGAAATGAGTGTGACTTAATGACGGCGCTTAGTTATAATCGTAGTCGGGAGCGTAAAGATTATC
 TTTGAGAATGTTGAAAGTACCAAACCCCTCCCAAAGTGGCTCAGGTCCCACCGCTTCTGTAAAAATTTGTC
 TCCCCCTACCCCAACAGTGTAAACACAGGAAAGTCGATTAGATCAAACATAACAGATGCTTTACAGC
 CCCCACCAAAAAGAAAAAAGTGGTCCGTTTTTTAGGTACTTTATTCAAAATCTTTTTTCTTTCTTTTATT
 GTAGGTGTAGCACTCTTACTTATCTTATACTAACTAAACCAACTTCTGTGAAAGTTCCTAATGTAGCAGG
 CACTAGTCTTAAAGTTGCCAAACAAGAACTGTATGATGTTGGGCTAAAAGTGGGTAAAATCAGGCAAATTTG
 AGAGTGATACGGTTGCTGAGGGAAATGTAGTTAGAACAGATCCTAAAGCAGGAACAGCTAAGAGGCAAGGC
 TCAAGCATTACGCTTTATGTGTCAATGGAAACAAAGGTTTTGACATGGAAAACACAAAGGACTAGATTA
 TCAAGAAGCTATGAATAGTTTGATAGAACTTATGGTGTTCAAAATCAAAAATCAAAAATGAGCGCATTTG
 TAACTAATGAATATCCTGAAAATACAGTCAATCAGTCAATCGCCAAGTGGGGTGATAAATTTAATCCAAAC
 GAAAGTCTAAAATTACGCTCAGTGTGCTGTTAGTGTATGATGATGATGATGATGATGATGATGATGATGATG
 TTATGCAGATGCAGTCAATACCTTAAACAGCTTTAGGTATAGATGCATCTAGAATAAAAAGCTTATGTGCCAA
 GCTCTAGCTCAGCAACGGGCTTTGTGCCAATTCATTCCTTAGTTCTAAAAGCTATTGTGTCAGTGGTCAATCT
 CCTTACTATGGAACGTCTTTGAGTCTGTCTGATAAAGGAGAGATTAGTCTTTACCTTTATCCAGAAGAAAC
 ACACCTTCTAGTAGCTCATCGAGTTCACCGTCAAGTTCAAACAGTTCCTTCAATAAATGATAGTACTGCAC
 CAGGTAGCAACACTGAATTAAGCCATCAGAAACTACTTCTCAAACACCTTAA

SEQ ID NO: 74 amino acid sequence comprising GAS 39

MDLILFLLVLLVLLGLGAYLLFKVNLQHQLAQTLEGNADNLSQDMTYQLDTANKQQLLELTLQLMNRQQAGL
 YQQLTDIRDVLHRSLSDSRDSRSDKRLEKINQQVNSLKNMQESNEKRLEKMRQIVEEKLEETLKNRHLHASF
 DSVSKQLESVNLKGLGEMRVAQDVGTLNKVLNKTGRVIGLDELQIQIIEDIMTSSQYEREFVTVSGSSER
 VEYAIKLPNGNGGGYIYLPIDSKFPLEDYRLEAYEYVGDKLAIEASRKALLAAIKRFAKDIHKYLNPPPE
 TTNFGVMFLPTEGLYSEVVRNASFFDSLRRREENIVVAGPSTLSALLNLSLVGFKTLNIQKNADDISKILGN
 VKLEFDKFGGLLAKAQKQMNTANNTLDQLISTRNVAIVRALNTVETYQDQATKSLNMPLEENEN

SEQ ID NO: 75 polynucleotide sequence encoding GAS 39

ATGGACCTTATCTTGTTCCTTTTGGTCTTGGTTCCTTTAGGTTTAGGGGCTTATCTGTTGTTCAAAGTCAA
 CGGCTTCAACATCAGCTTGCCCAAACCTTAGAAGGCAACGCGGATAATTTGTCTGACCAAATGACCTACC
 AGTTGGATACAGCTAACAAACAACAATTTGTTAGAGCTAACACAGCTGATGAACCGACAACAAGCAGGCCCTT
 TACCAACAATTAACAGATATTCGTTGACGCTTGCACCGTAGTTTGTCTGATAGTAGGGACCGGCTGACAAA
 ACGCTTAGAAAAAATTAACCAGCAGGTCAACCAATCGCTCAAAAATATGCAAGAATCTAACGAAAAACGTT
 TGGAGAAAATGCGCCAGATCGTTGAAGAAAAAATGGAAGAAACCTTAAAAAATCGTCTGCACGCCCTCTTTC
 GATTCGTATCCAAGCAACTAGAAAAGTGTCAATAAAGGCTTGGGAGAAATGCGTAGCGTGGCTCAAGATGT
 GGGTACTTTAAATAAGGTTTTGTCCAATACCAAAACACGAGGCATTTTAGGGGAACTTCAACTAGGCCAAA
 TCATTGAGGATATCATGACATCAAGCCAGTACGAAAGAGAATTTGTAACGGTTAGTGGTTCAGTGAACGC
 GTAGAATATGCGATTAAGCTCCAGGAAATGGTCAAGGCGGTTATATTTACCTACCGATTGACTCAAATTT
 CCTCTTGAAGATTATTACCGATTAGAAAGATGCTTACGAAGTTGGTGATAAACTGGCCATCGAGGCTAGCC
 GAAAAGCACTTCTGGCAGCTATCAAACGCTTTGCCAAAGACATTCATAAAAAGTACTTGAACCCCCAGAG
 ACGACCAATTTGGAGTTATGTTCTTACCAACAGAAGGCTTTTATTTCAGAAGTGGTCAGAAATCGCTCTTT
 CTTTGATAGCCTTCGTCGGGAAGAAAAATATTTGTGGTTCAGGCCCTTCGACCCTGTCTGCTTTGCTGAATT
 CCTTATCTGTTGGTTTTCAAGACCTTAAATATCCAAAAAATGCTGATGACATCAGTAAAAATTTAGGCAAT
 GTCAGTTAGAATTCGATAAATTTGGCGGCTGCTTGCCAAGGCTCAAAAACAATGAATACAGCTAATAA
 TACGCTGGATCAGCTCATTTCACAACAGGACAAATGCCATTGTTTCGAGCCTTGAATACCGTTGAACTTATC
 AAGACCAAGCAACAATCTCTCTTGAACATGCCCTTATTAGAAGAGGAAAAATAATGAAAAATTA

SEQUENCE LISTING

SEQ ID NO: 76 amino acid sequence comprising GAS 42

MTKEKLVAFSQAHAEPAWLQERRLAALAEI PNLELPTIERVKFHRWNLGDGTLTENESLASVPDFIAIGDN
PKLVQVGTQTVLEQLPMALIDKGVVFSDFYTALEEIPEVIEAHFGQALAFDEDKLAAAYHTAYFNSSAAVLYV
PDHLEITTPIEAIFLQDSDDVFPFNKHLVVIAGKESKFTYLERFESIGNATQKISANISVEVIAQAGSQIK
FSAIDRLGPSVTTYISRRGRLEKDANIDWALAVMNEGNVIADFSDSLIGQGSQADLKVVAASSGRQVQGID
TRVTNYGQRTVGHILQHGVILERGLTFNGIGHILKDAKGADAQQESRVLMLSDQARADANPILLIDENEV
TAGHAASIGQVDPEDMYLMSRGLDQETAERLVIRGFLGAVIAEIPISVRQEI IKVLDEKLLNR

SEQ ID NO: 77 polynucleotide sequence encoding GAS 42

ATGACAAAAGAAAACTAGTGGCTTTTTCGCAAGCCCACGCTGAGCCTGCTTGGCTGCAAGAACGGCGTTT
AGCGGCATTAGAAGCCATTCCAAATTTGGAATTACCAACCATCGAAAAGGGTTAAATTTCCACCGTTGGAATC
TAGGAGATGGTACCTTAACAGAAAAATGAAAGTCTAGCTAGTGTTCAGATTTTATAGCTATTGGAGATAAC
CCAAAGCTTGTTCAGGTAGGCACGCAAAACAGTCTTAGAACAGTTACCAATGGCGTTAATTGACAAGGGAGT
TGTTTTTCAGTGATTTTTTATACGGCGCTTGAGGAAATCCCAGAAGTAATTGAAGCTCATTTTGGTCAGGCAT
TAGCTTTTTGATGAAGACAAACTAGCTGCCTACCACACTGCTTATTTTAATAGCGCAGCCGTGCTCTACGTT
CCTGATCACTTGGAAATCACAACCTCCTATTGAAGCTATTTTCTTACAAGATAGTGACAGTGACGTTCCTTT
TAACAAGCATGTTCTAGTGATTGCAGGAAAAGAAAGTAAGTTCACCTATTTAGAGCGTTTTGAATCTATTG
GCAATGCCACTCAAAAGATCAGCGCTAATATCAGTGTAGAAGTGATTGCTCAAGCAGGCAGCCAGATTAAA
TTCTCGGCTATCGACCGCTTAGGTCCTCAGTGACAACCTATATTAGCCGTCGAGGACGTTTAGAGAAGGA
TGCCAACATTGATTGGGCCTTAGCTGTGATGAATGAAGGCAATGTCATTGCTGATTTTGACAGTGATTTGA
TTGGTCAGGGCTCACAAGCTGATTTGAAAGTTGTTGCAGCCTCAAGTGGTCGTCAGGTACAAGGTATTGAC
ACGCGCTGACCAACTATGGTCAACGTACGGTCGGTCATATTTACAGCATGGTGTGATTTTGAACGTTG
CACCTTAACGTTTAAACGGGATTTGGTCATATTTCAAAGACGCTAAGGGAGCTGATGCTCAACAAGAAAGCC
GTGTTTTGATGCTTTCTGACCAAGCAAGAGCCGATGCCAATCCAATCCTCTTAATTGATGAAAATGAAGTA
ACAGCAGGTTCATGCAGCTTCTATCGGTCAGGTTGACCCTGAAGATATGTATTACTTGATGAGTCGAGGACT
GGATCAAGAAACAGCAGAACGATTGGTTATTAGAGGATTCCTAGGAGCGGTTATCGCTGAAATTCCTATTC
CATCAGTCCGCCAAGAGATTATTAAGTTTTAGATGAGAAATTGCTTAATCGTTAA

SEQ ID NO: 78 amino acid sequence comprising GAS 58

MKWSGFMKTKSKRFLNLAATLCLALLGTTLLMAHPVQAEVISKRDYMTFRFGLGDLEDDSANYPNLEARYKG
YLEGYEKGLKGDDIPERPKIQVPEDVQPSDHGDYRDGYEEGFGEGQHKRDPLETEAEDDSQGGRQEGRQGH
QEGADSSDLNVEESDGLSVIDEVVGVIYQAFSTIWTYLSGLF

SEQ ID NO: 79 polynucleotide sequence encoding GAS 58

ATGAAATGGAGTGGTTTTTATGAAAACAAAATCAAACGCTTTTTTAAACCTAGCAACCCTTTGCTTGGCCCT
ACTAGGAACAACCTTTGCTAATGGCACATCCCGTACAGGCGGAGGTGATATCAAAAAGAGACTATATGACTC
GCTTCGGGTTAGCGGATTTAGAAGATGATTCAGCTAACTATCCTTCAAATTTAGAAGCTAGATATAAAGGA
TATTTAGAGGGATATGAAAAGGCTTAAAAGGAGATGATATACCCGAACGGCCCAAGATTCAGGTTCCCTGA
GGATGTTTCAGCCATCTGACCATGGCGACTATAGAGATGGTTATGAGGAAGGATTTGGAGAAGGACAACATA
AACGTGATCCATTAGAAAACAGAAAGCAGAAGATGATTCTCAAGGAGGACGTCAGAAGGACGTCAGGACAT
CAAGAAGGAGCAGATTCTAGTGATTTGAACGTTGAAGAAAGCGACGGTTGTCTGTTATTGATGAAGTAGT
TGGAGTAATTTATCAAGCATTAGTACTATTTGGACATACTTAAGCGGTTTGTCTAA

SEQ ID NO: 80 amino acid sequence comprising N-terminal leader sequence of GAS 58

MKWSGFMKTKSKRFLNLAATLCLALLGTTLLMA

SEQ ID NO: 81 amino acid sequence comprising a fragment of GAS 58 where the N-terminal leader sequence is removed

HPVQAEVISKRDYMTFRFGLGDLEDDSANYPNLEARYKGYLEGYEKGLKGDDIPERPKIQVPEDVQPSDHG
DYRDGYEEGFGEGQHKRDPLETEAEDDSQGGRQEGRQGHQEGADSSDLNVEESDGLSVIDEVVGVIYQAFS
TIWTYLSGLF

SEQ ID NO: 82 amino acid sequence comprising GAS 290

SEQUENCE LISTING

MKHILFIVGSLREGSFNHQLAAQAQKALEHQAVVSYLNNKQDIEANAPLPVVDARQAVQSADAIW
IFTPVYVNF S I P G S V K N L L D W L S R A L D L S D P T G P S A I G G K V V T V S S V A N G G H D Q V F D Q F K A L L P F I R T S V A G
E F T K A T V N P D A W G T G R L E I S K E T K A N L L S Q A E A L L A A I

SEQ ID NO: 83 polynucleotide sequence encoding GAS 290

ATGAAACATATTTTATTTATTTGTTGGCTCGCTTCGTGAAGGGTCTTTTAAACCATCAATTAGCGGCTCAAGC
ACAAAAGCTCTGGAACATCAAGCAGTTGTATCTTACTTAAATGGAAAGACGTTCCCTGTTTTGAATCAAG
ATATCGAAGCTAATGCACCTTTACCAGTTGTTGACGCTCGTCAAGCTGTTTCAGTCAGCGGATGCTATCTGG
ATTTTTACACCAGTTTACAACCTTCTCTATTTCCAGGTTCTGTTAAAAACCTGCTAGACTGGTGTCTCGTGC
TCTTGATTTGCTGATCCGACGGGCCATCTGCTATTGGCGGTAAGGTGGTTACGGTCTCTTCAGTTGCAA
ATGGCGGGCATGATCAAGTATTTGATCAGTTTAAAGCACTATTGCCGTTTATCCGAACCTTCAGTAGCAGGA
GAGTTTACAAAAGCAACTGTGAATCCTGATGCCTGGGGAACAGGAAGGCTTGAGATTTCAAAGAGACAAA
AGCAAACCTTGCTATCTCAGGCAGAGGCTCTTTTAGCGGCTATTTAG

SEQ ID NO: 84 amino acid sequence comprising GAS 511

MTDVSRIKLEARDQGRLLTLDYANLIFDDFMELHGDRHFSDDGAIVGGGLAYLAGQPVTVIGIQKGNLQDN
LARNFGQPNPEGYRKALRLMKQAEKFRPVVTFINTAGAYPGVGAEBERGQGEAIAKNLMEMSDLKVPILIAI
IIGEGSGGALALAVADQVWMLENTMYAVLSPEGFASILWKDGSRAEAEMLKITAGELYKMGIVDRIIP
EHGYFSSEIVDIKANLIEQITSLQAKPLDQLLDERYQRFKRY

SEQ ID NO: 85 polynucleotide sequence encoding GAS 511

ATGACAGATGTATCAAGAATTTTAAAAGAAGCGGTGATCAAGGGCGTTTAAACAACCTTTGGATTACGCCAA
CCTTATTTTCGATGACTTTATGGAACGTCATGGCGATCGCCATTTTTCAGATGATGGTGCCATTGTAGGTG
GCCTAGCTTATTTGGCGGGACAACCTGTTACGGTCATTGGTATTCAAAAGGTAAGAATTTACAGGATAAT
TTGGCAAGGAATTTGGCCAGCCCAATCCAGAAGTTATCGTAAAGCTTTGCGCCTTATGAAACAGGCAGA
AAAATTTGGACGACCAGTTGTTACGTTTATCAATACTGCAGGAGCCTATCCAGGTGTCGGTGCAGGAAGAAC
GAGGACAGGGTGAGGCCATTGCTAAAAATTTGATGAAATGAGTGATCTCAAGGTTCCATTATCGCCATC
ATTATTGGTGAAGGAGGCTCTGGTGGTGCATTAGCCTTAGCGGTTGCCGATCAGGTCTGGATGCTTGAAAA
TACTATGTATGCGGTTCTTAGCCAGAAAGCTTTGCTTCTATTTTATGGAAGGATGGTTCAAGGGCGACCG
AGGCCGCTGAATGATGAAAATCACAGCGGGTGAACCTTACAAAATGGGAATAGTAGACCGTATTATTCCA
GAACATGGTTATTTTTCAAGTGAATCGTTGACATCATCAAAGCTAACCTCATCGAACAAATAACCAGTTT
GCAAGCTAAGCCATTAGACCAATTATTAGATGAGCGCTACCAACGCTTTCGTAAATATTA

SEQ ID NO: 86 amino acid sequence comprising GAS 533

MAITVADIRREVKEKNVTFRLRMFTDIMGVMKNVEIPATKEQLDKVLSNKVMFDGSSIEGFVVRINESDMYL
YPLDLDTWIVFPWGDENGAVAGLICDIYTAEGKPFAGDPRGNLKRALKHMNEIGYKSFNLGPEPEFFLFKMD
DKGNPTLEVNNDNGYFDLAPIDLADNTRREIVNILTKMGFEVEASHHEVAVGQHEIDFKYADVLKACDNIQ
IFKLVVKTIAREHGLYATFMAKPKFGIAGSGMHCNMSLFDNQGNNAFYDEADKRGMLSEDAYYFLGGLMK
HAYNYTALTNPTVNSYKRLVPGYEAPVYVAVAGSNRSPILIRVPASRGMGTRLELRSVDPTANPYLALAVLL
EAGLDGIINKIEAPEPEANIYTMTEERNEAGIIDLPSTLHNALKALQKDDVVQKALGYHIYTNFLEAKR
IEWSSYATFVSWQWEIDHYIHNY

SEQ ID NO: 87 polynucleotide sequence encoding GAS 533

ATGGCAATAACAGTAGCTGACATTCGTGCTGAAGTCAAAGAAAAAATGTAACGTTTCTTCGCTTGATGTT
CACTGATATCATGGGCGTTATGAAAAATGTGGAGATTCCTGCAACTAAAGAACAGTTAGACAAAAGTATTGT
CTAACAAGGTTATGTTTGTATGGTTCATCTATCGAAGGTTTGTACGGATCAATGAGTCAGATATGTACCTT
TACCCCGATTTAGACACTTGGATTGTTTTTCCCTGGGGAGATGAAAATGGAGCAGTTGCAGGTTAATTTG
TGATATTTATACAGCAGAAGGAAAGCCTTTTGCAGGAGATCCTAGAGGAAATTTAAAAGAGCCCTGAAAC
ACATGAACGAGATCGGCTACAAATCATTAAATCTTGGACCAGAACCAGAATTTTTCTTTTAAAGATGGAT
GATAAAGTAAATCCGACACTTGAAGTTAACGATAATGGTGGTTATTTTGATTTAGCGCCAATTGACTTAGC
AGACAACACGCGCCGTGAAATTTGTAATATTTTAAAGAAAATGGGTTTGAAGTGAAGCTAGTCATCATG
AAGTGGCTGTTGGTCAACATGAGATTGATTTTAAATATGCAGATGTTTTGAAAGCTTGTGATAATATTCAA
ATTTTAAAGCTAGTTGTA AAAACGATTGCCCGTGAACATGGACTTTATGCTACTTTTCATGGCTAAACCAA
ATTTGGAATAGCTGGATCAGGGATGCACGTAAACATGCTTTTGTGATAACCAAGGTAATAATGCTTTT
ATGATGAAGCTGATAAGCGAGGGATGCAGTTATCAGAAGATGCTTATTATTCTTTGGGAGGACTAATGAAG
CATGCTTATAACTACACTGCTATCACTAACCTACAGTGAATTTTATAAACGATTAGTTCAGGTTATGA

SEQUENCE LISTING

GGCACCTGTTTATGTCGCTTGGGCTGGAAGTAATCGTTCACCGCTTATCCGTGTTCCAGCATCACGTGGTA
 TGGGAACGCGTTTGGAGTTACGTTTCGGTTGATCCGACAGCTAATCCTTATTTAGCCTTGGCTGTTCTCTTG
 GAAGCTGGATTAGATGGTATCATTAACAAAATTGAAGCTCCAGAACCCTTGAAGCTAACATTTATACCAT
 GACAATGGAAGAACGAAATGAAGCAGGCATTATTGATTTGCCATCAACGCTTCATAATGCCTTAAAAGCTC
 TTCAAAAAGATGATGTGGTACAAAAGGCAC TAGGTTACCATATCTACACTAATTTCTTAGAAGCAAAACGA
 ATTGAATGGTCTTCCTATGCAACTTTTGTTCCTCAATGGGAAAATTGACCATTATATTTACATAATTATTAG

SEQ ID NO: 88 amino acid sequence comprising GAS 527

MTEISILNDVQKIIIVLDYGSQYNQLIARRIREFGVFSELKSHKITAQELREINPIGIVLSGGPNSVYADNA
 FGIDPEIFELGIPILGICYMQLITHKLGKVVVPAQAGNREYQSTLHLRETSKLFSGTPQEQLVLM SHG
 DAVTEIPEGFHLVGDSDNDCPYAAIENTEKNLYGIQFHPEVRHSVYVNDILKNFAISICGARGDWSMDNFID
 MEIAKIRETVGDRKVLGLSGGVDSSVVGVLQKAIQDQLTCIFVDHGLLRKDEGDQVMGMLGGKFGFLNII
 RVDASKRFLDLLADVDEPEKRRKIIGNEFVYVDFDEASKLKGVDFLAOGTLYTDIIESGTETAQTIKSHHN
 VGGLPEDMQFELIEPLNLFKDEVRALGIALGMPEEIVWRQPFPGPLAIRVMGAIITEEKLETVRES DAIL
 REEIAKAGLDRDVWQYFTVNTGVRVGVMDGRTYDYTIAIRAITSIDGMTADFAQLPVDVLKKI STRIVN
 EVDHVNRIVYDITSKPPATVEWE

SEQ ID NO: 89 polynucleotide sequence encoding GAS 527

ATGACTGAAATTTCAATTTTGAATGATGTTCAAAAAATTATCGTTCCTTGATTATGGTAGCCAGTACAATCA
 GCTTATTGCTAGACGTATTCGAGAGTTTGGTGTTCCTCCGAATAAAAAGCCATAAAAATCACCGCTCAAG
 AACTTCGTGAGATCAATCCCATAGGTATCGTTTTATCAGGAGGGCCTAACTCTGTTTACGCTGATAACGCC
 TTTGGCATTGACCCCTGAAATCTTTGAACTAGGGATTCCGATTCCTGGTATCTGTTACGGTATGCAATTAAT
 CACCCATAAATTAGGTGGTAAAGTTGTTCCCTGCTGGACAAGCTGGTAATCGTGAATACGGTCAGTCAACCC
 TTCATCTTCGTGAAACGTCAAAATTAATTTTCAGGCACACCTCAAGAACAACTCGTTTTGATGAGCCATGGT
 GATGCTGTACTGAAATTCGAGAAGGTTCCACCTTGTTGGGAGACTCAAATGACTGTCCTATGCAGCTAT
 TGAATAACTGAGAAAAACCTTTACGGTATTTCAGTTCCACCCAGAAGTGAGACACTCTGTTTATGGAAATG
 ACATTTCTAAAAACTTTGCTATATCAATTTGTGGCGCGCTGGTATGGTCAATGGATAATTTTATTGAC
 ATGGAAATGCTAAAATTCGTGAAACTGTAGGCGATCGTAAAGTTCCTTAGGCTTTCTGGTGGAGTTGA
 TTCTTCAGTTGTTGGTGTTCCTACTTCAAAAAGCTATCGGTGACCAATTAAC TTGTATTTTCGTTGATCACC
 GTCTTCTTCGTAAAGACGAGGGCGATCAAGTTATGGGAATGCTTGGGGGCAAATTTGGCCATAAATATTATC
 CGTGTGGATGCTTCAAAACGTTTCTTAGACCTTCTTGCAGACGTTGAAGATCCTGAGAAAAAACGTA AAAAT
 TATTGGTAATGAATTTGTCATGTTTTTGTATGATGAAGCCAGCAAATTA AAAGGTGTTGACTTCCTTGCCC
 AAGGAACACTTTTACTGATATCATTGAGTCAGGAACAGAAACTGCTCAAACCATCAAATCACATCACAAT
 GTGGGTGGTCTTCCCGAAGACATGCAGTTTGAATTGATTGAGCCCTTAAACACTCTTTTCAAAGATGAAGT
 TCGAGCGCTTGGAAATCGCTCTTGGAAATGCCTGAAGAAATTTGTTGGCGCCAACCATTTCCAGGTCTGGAC
 TTGCTATCCGTGTCATGGGAGCAATTAAGTGAAGAAAAACTTGAACCGTTCGCGAATCAGACGCTATCCTT
 CGTGAAGAAATGCTAAGGCTGGACTTGATCGTGACGTTGGCAATACTTTACAGTTAACACAGGTGTCCG
 TTCTGTAGGCGTCATGGGAGATGGTCGTACTTATGATTATACCATCGCCATTCGTGCTATTACGTCTATTG
 ATGGTATGACAGCTGACTTTGCTCAACTTCCTTGGGATGCTTGAAAAAATCTCAACACGTATCGTAAAT
 GAAGTTGACCACGTTAACCGTATCGTCTACGACATCAAGTAAACCACCCGCAACAGTTGAATGGGAATA
 A

SEQ ID NO: 90 amino acid sequence comprising GAS 294

MSQSTATYINVIGAGLAGSEAAAYQIAKRGI PVKLYEMRGVKATPQHKTTNFAELVCSNSFRGDSL TNAVGL
 LKEEMRRLDSIIMRNGEANRVPAGGAMAVDREGYAESVTAELNHPLIEVIRGEITEIPDDAITVIATG PL
 TSDALAEKIHALNGGDGFYFYDAAAPIIDKSTIDMSKVYLSRYDKGEAYLNC PMTKEEFMAFHEALTTA
 EEAPLNAFEKEKYFEGCMPIEVMAKRGIKTMLYGPMKPVGLEYPDDYTGPRDGEFKTPYAVVQLRQDNAAG
 SLYNIVGFQTHLKWGEQKRVFQMI PGLENAEFVRYGVMHRNSYMDSPNLLTETFQSRSNPNLFFAGQMTGV
 EGYVESAAAGLVAGINAARLFKREELIFPQTTAIGSLPHYVTHADSKHFQPMNVNFGI I KELEGPRIRDK
 KERYEAIASRALADLDTCLASL

SEQ ID NO: 91 polynucleotide sequence encoding GAS 294

TTGTCCTCAATCAACTGCAACTTATATTAATGTTATTGGAGCTGGGCTAGCTGGTTCTGAAGCTGCCTATCA
 GATTGCTAAGCGCGGTATCCCCGTTAAATTGTATGAAATGCGTGGTGTCAAAGCAACACCGCAACATAAAA
 CCACTAATTTTGCCGAATTGGTCTGTTCCAACCTCATTTCGTGGTGATAGCTTAACCAATGCAGTCGGTCTT
 CTCAAAAGAAGAAATGCGGCGATTAGACTCCATTATATGCGTAATGGTGAAGCTAACCGCGTACCTGCTGG

SEQUENCE LISTING

GGGAGCAATGGCTGTTGACCGTGAGGGGTATGCAGAGAGTGTCACTGCAGAGTTGGAAAATCATCCTCTCA
 TTGAGGTCATTTCGTGGTGAAATACAGAAATCCCTGACGATGCTATCACGGTTATCGCGACGGGACCGCTG
 ACTTCGGATGCCCTGGCAGAAAAATTCACGCGCTAAATGGTGGCGACGGATTCATTTTTTACGATGCAGC
 AGCGCCTATCATTTGATAAATCTACCATTGATATGAGCAAGGTTTACCTTAAATCTCGCTACGATAAAGCG
 AAGCTGCTTACCTCAACTGCCCTATGACCAAAGAAGAATTCATGGCTTCCATGAAGCTCTGACAACCGCA
 GAAGAAGCCCCGCTGAATGCCCTTTGAAAAAGAAAAGTATTTTGAAGGCTGTATGCCGATTGAAGTTATGGC
 TAAACGTGGCATTAATAACCATGCTTTTATGGACCTATGAAACCCGTTGGATTGGAATATCCAGATGACTATA
 CAGGTCCTCGCGATGGAGAAATTAATAACGCCATATGCCGTCGTGCAATTCGCTCAAGATAATGCAGCTGGA
 AGCCTTTATAATATCGTTGGTTTCCAAACCCATCTCAAATGGGGTGAGCAAAAACGCGTTTTTCCAAATGAT
 TCCAGGGCTTGAATAATGCTGAGTTTGTCCGCTACGGCGTCATGCATCGCAATTCCTATATGGATTACCAA
 ATCTTTTAACCGAAACCTTCCAATCTCGGAGCAATCCAAACCTTTTCTTTGACGGTCAGATGACTGGAGTT
 GAAGTTATGTCGAATCAGCTGCTTCAGGTTTAGTAGCAGGAATCAATGCTGCTCGTTTGTTCAAAAGAGA
 AGAAGCACTTATTTTCCCTCAGACAACAGCTATTGGGAGTTTGCCTCATTATGTGACTCATGCCGACAGTA
 AGCATTTCCAACCAATGAACGTCAACTTTGGCATCATCAAAGAGTTAGAAGGCCACGCATTCGTGACAAA
 AAAGAACGTTATGAAGCTATTGCTAGTCGTGCTTTGGCAGATTTAGACACCTGCTTAGCGTCGCTTTAA

SEQ ID NO: 92 amino acid sequence comprising GAS 253

MPKKILFTGGGTGVHVTLLNLILIPKFIKDGWEVHYIGDKNGIEHTEIEKSGLDVTFHAIATGKLRRYFSWQ
 NLADVFKVALGLLQSLFIVAKLRPQALFSKGGFVSVPPVVAAKLLGKPVFIHESDRSMGLANKIAYKFATF
 MYTTFEQEDQLSKVKHLGAVTKVFKDANQMPSTQLEAVKEYFSRDLKTLLEF IGGSAGAHVFNQFISDHPE
 LKQRYNIINITGDEPHLNELSSHLYRVDYVTDLYQPLMAMADLVVTRGGSNTLFE LLAMAKLHLIVPLGKEA
 SRGDQLENATYFEKRGYAKQLQEPDLTLHNFQAMADLFEHQADYEATMLATKEIQSPDFYDLRADISS
 AIKEK

SEQ ID NO: 93 polynucleotide sequence encoding GAS 253

ATGCCTAAGAAGATTTTATTACAGGTGGTGGAACTGTAGGTCATGTCACCTTGAACCTCATTCTCATAACC
 AAAATTTATCAAGGACGGTTGGGAAGTACATTATATTGGTGATAAAAAATGGCATTGAACATACAGAAATG
 AAAAGTCAGGCCCTTGACGTGACCTTTCATGCTATCGCGACAGGCAAGCTTAGACGCTATTTTTTCATGGCAA
 AATCTAGCTGATGTTTTTAAGGTTGCACCTGGCCTCCTACAGTCTCTCTTTATTGTTGCCAAGCTTCGCCC
 TCAAGCCCTTTTTTCCAAAGGTGGTTTTTGTCTCAGTACCGCCAGTTGTGGCTGCTAAATTGCTTGGTAAAC
 CAGTCTTTATTTCATGAATCAGATCGGTCAATGGGACTAGCAAACAAGATTGCCTACAAATTTGCAACTACC
 ATGTATACCACTTTTGAGCAGGAAGACCAGTTGTCTAAAGTTAAACACCTTGGAGCGGTGACAAAAGGTTTT
 CAAAGATGCCAACCAATGCCATCAACTCAGTTAGAGGGCGGTGAAAGAGTATTTTAGTAGAGACCTTAA
 AAACCTCTTGTATTATTTGGTGGTTTCGGCAGGGGCGCATGTGTTTAAATCAGTTTATTAGTGATCATCCAGAA
 TTGAAGCAACGTTATAATATCATCAATATTACAGGAGACCCTCACCTTAATGAATTGAGTTCTCATCTGTA
 TCGAGTAGATTATGTTACCGATCTCTACCAACCTTTGATGGCGATGGCTGACCTTGTAGTGACAAGAGGGG
 GCTCTAATACACTTTTTGAGCTACTGGCAATGGCTAAGCTACACCTCATCGTTTCTTGGTAAAGAAGCT
 AGCCGTGGCGATCAGTTAGAAAATGCCACTTATTTGAGAAGAGGGGCTACGCTAAACAATTACAGGAACC
 TGATTTAACTTTGCATAATTTTATGATCAGGCAATGGCTGATTTGTTTGAACATCAGGCTGATTATGAGGCTA
 CTATGTTGGCAACTAAGGAGATTCAGTACCCGACTTCTTTTATGACCTTTTGGAGAGCTGATATTAGCTCC
 CGGATTAAGGAGAAGTAA

SEQ ID NO: 94 amino acid sequence comprising GAS 529

MCGIVGVVGNRNATDILMQGLEKLEYRGYDSAGIFVANANQTNLIKSVGRIADLRKIGIDVAGSTGIGHT
 RWATHGQSTEDNAHPHTSQTGRFVLVHNGVIENYLHIKTEFLAGHDFKQTDTEIAVHLIGKFVEEDKLSV
 LEAFKKSLSIIEGSYAFALMDSQATDTIYVAKNKSPLLI GLGEGYNMVCSDAMAMIRETSEFMEIHDKELV
 ILTKDKVTVTDYDGKELIRDSYTAELDLSDIGKGYPFYMLKEIIDEQPTVMRQLISTYADETGNVQVDPAI
 ITSIQEADRLYILAAGTSYHAGFATKNMLEQLTDTPVELGVASEWGYHMPLLSKKPMFILLSQSGETADSR
 QVLVKANAMGIPSLTVTNVPGSTLSREATYTMLIHAGPEIAVASTKAYTAQIAALAF LAKAVGEANGKQEA
 LDFNLVHEL SLVAQSI EATLSEKDLVAEKVQALLATTRNAFYI GRGNDYVAMEAALKLKEISYIQCEGFA
 AGELKHGTISLIEEDTPVIALI SSSQLVASHTRGNIQEVAARGAHLVTVVEEGLDREGDDI IVNKVHPFLA
 PIAMVIP TQLIAYYASLQRGLDVKPRNLAKAVTVE

SEQ ID NO: 95 polynucleotide sequence encoding GAS 529

ATGTGTGGAATTGTTGGAGTTGTTGGAAATCGCAATGCAACGGATATTTTAAATGCAAGGCCTTGAAAAGCT
 TGAATACCGGGGTTATGATTCAGCAGGAATTTTTGTGGCTAATGCCAATCAACAACCTTGATTAATCAG

SEQUENCE LISTING

TGGGGCGGATTGCTGATTTGCGTGCCAAAGATTGGCATTGATGTTGCTGGTTCAACAGGGATTGGTCACACC
 CGTTGGGCAACGCATGGCCAATCAACAGAGGATAATGCCCATCCTCACACGTCACAACTGGACGTTTTGT
 ACTTGTTCATAATGGTGTGATTGAAAATTACCTTCACATTA AAAACAGAGTTCC TAGCTGGACATGATTTTA
 AGGGGCAGACAGATACTGAGATTGCAGTACACTTGATTGGAAAAATTTGTGGAAGAAGACAAGTTGTCTAGTA
 CTGGAAGCTTTTAAAAAATCTTTAAGCATTATTGAAGGTTCC TACGCCTTTGCATTAATGGATAGCCAAGC
 AACTGATACTATTTATGTGGCTAAAAACAAGTCTCCATTGTTGATGGACTTGGTGAAGGTTACAACATGG
 TTTGTTTCAGATGCCATGGCCATGATTTCGTGAAACCAGTGAATTTATGGAAATTCATGATAAGGAGCTAGTT
 ATTTTAACCAAAGATAAGGTAACGTGTTACAGACTACGATGGTAAAGAGCTGATACGAGATTCC TACTGC
 TGAATTAGACTTATCTGATATGGCAAAGGGACTTATCCTTTCTATATGCTGAAAGAAATTGATGAGCAAC
 CAACCGTAATGCGTCAATTAATTTCAACTTATGCAGATGAAACTGGTAACGTACAGGTTGATCCGGCTATC
 ATTACCTCTATCCAAGAGGCTGACCGTCTTTATATTTTAGCGGCAGGGACTTCC TACCATGCTGGTTTTGC
 AACAAAAATATGCTTGAGCAATTGACAGATAACCCAGTTGAGTTGGGCGTGGCTTCTGAGTGGGGTTACC
 ACATGCCCTCTGCTTAGCAAGAAACCAATGTTTATTCTACTAAGCCAATCAGGAGAAACCCGAGATAGTCGT
 CAAGTTT TAGTAAAGGCAAATGCTATGGGCATTCAGGTTGACAGTAAC TAACGTTCCAGGATCAACCTT
 ATCACGTGAAGCAACATACACCATGTTGATTTCATGCTGGACCTGAAATTTGCTGTTGCGTCTACAAAAAGCTT
 ACATGCAAAAATTTGCTGCCCTTGCCTTTTGGCTAAGGCAGTTGGT GAGGCAAATGGTAAGCAAGAAGCT
 CTTGACTTTTAACTTGGTACATGAGTTGTCAATTTGGTTGCCCAATCTATTGAGGCGACTTTGTCTGAAAAAGA
 TCTCGTGGCAGAAAAGGTTCAAGCTTTGCTAGCTACTACTCGTAATGCTTTTTACATCGGGCGTGGCAATG
 ATTATTACGTTGCGATGGAAGCTGCTTTGAAATTA AAAAGAGATTTCTTATATTCAATGCGAAGGCTTTGCG
 GCTGGTGAATTGAAACATGGAACATTT CATTAATTGAGGAGGACACGCCAGTAATCGCTTTAATATCGTC
 TAGTCAGTTGGTTGCCCTCTCATACGCGTGGTAATATTCAAGAAGTTGCTGCCCGTGGGGCTCATGTTTTAA
 CAGTTGTGGAAGAAGGGCTTGACCGTGAGGGAGATGACAT TATTGTCAATAAGGTT CATCCTTTCTAGCC
 CCGATTGCTATGGTCATTTCAACTCAACTGATTGCTTACTACGTTT CATTACAACGTGGACTTGATGTTGA
 TAAGCCACGTAATTTGGCTAAAGCTGTAACAGTAGAATAA

SEQ ID NO: 96 amino acid sequence comprising GAS 45

VTFMKS^KSWLA^AAVSV^AILSV^ASALAA^ACGN^KKNAS^GGGSEAT^KTYKY^VFVND^PPKSLDY^ILTN^GGGT^TDVIT^QMVD
 GLLE^NDEY^GNLVP^SLAKDW^KVSKD^GLYTY^TTLRD^GVSWY^TADGEE^YAPV^TAE^DFVT^GLKHA^VDD^KSDAL^V
 VEDS^IKNL^KAYQ^NGEV^DFK^EVGV^KALDD^KTVQ^YTLN^KPES^YWNS^KTTY^SVLF^PVNA^KFL^KSK^GKDF^GTDP
 SSIL^VNGAY^FLSA^FTSK^SSM^EFHK^NENY^WDA^KNV^GIES^VKL^TYS^DGS^DPG^SFY^KNF^DKGE^FSVAR^LYP^NDP
 TYK^SAK^NYAD^NITY^GML^TGD^IRHL^TWNL^NRT^SFK^NTK^KDP^AQ^QDAG^KKAL^NNK^DFR^QAI^QFA^FDRAS^FQA
 QTAG^QDA^KT^KAL^RNML^VPP^TFV^TIG^ESDF^GSE^VEK^EMA^KL^GDE^WKDV^NLADA^QDG^FYN^PEK^AKA^EFA^KAK^E
 AL^TAE^GV^TFP^VQ^LDY^PVD^QANA^ATV^QE^AQ^SFK^SQ^SVE^ASL^GKEN^VIV^NVLE^TET^STHE^AQ^GFYA^ET^PEQ^QDY
 DI^ISS^WVDY^QDR^PTY^LDI^MSP^VGG^SVI^QKL^GIK^AQ^NKD^VVAA^GLD^TY^QTL^LDE^AAAIT^DDND^ARY^K
 AYAK^AQAY^LTD^NAV^DIP^VVAL^GGT^PRV^TKA^VPF^SGG^FSW^AGS^KG^PLAY^KM^KLQ^DKP^VTV^KQY^EKA^KE^KW^M
 KAK^ASN^AKYA^EKL^ADH^VE^K

SEQ ID NO: 97 polynucleotide sequence encoding GAS 45

GTGACTTTTATGAAGAAAAGTAAATGGTTGGCAGCTGTAAGTGTTCGATCTTGTCTAGTATCCGCTTTGGC
 AGCTTGTGGTAATAAAAATGCTTCAGGTGGCTCAGAAAGCTACAAAACCTACAAGTACGTTTTTGTAAACG
 ATCCAAAATCATGGATTATATTTTACTAATGGCGGTGGAACGACTGATGTGATAACACAAATGGTTGAT
 GGTCTTTTGGAAAACGATGAGTATGGTAATTTAGTACCATCACTTGCTAAAGATTGGAAGGTTTCAAAGA
 CGGTCTGACTTATACTTATACTCTTCGCGATGGTGTCTCTTGGTATACGGCTGATGGTGAAGAATATGCC
 CAGTAACAGCAGAAGATTTTGTGACTGGTTTGAAGCACGCGGTTGACGATAAATCAGATGCTCTTTACGTT
 GTTGAAGATTCAATAAAAAACTTAAAGGCTTACCAAATGGTGAAGTAGATTTTAAAGAAGTTGGTGTCAA
 AGCCCTTGACGATAAAACTGTTTCACTATACTTTGAACAAGCCTGAAAGCTACTGGAATTCAAAACAACCTT
 ATAGTGTGCTTTTCCAGTTAATGCGAAATTTTGAAGTCAAAGGTAAAGATTTTGGTACAACCGATCCA
 TCATCAATCCTTGTAAATGGTGTACTTCTTGGAGCGCTTACCTCAAATCATCTATGGAATTCATAA
 AAATGAAAAC^TACTGGGATGCTAAGAATGTTGGGATAGAATCTGTTAAATTGACTTACTCAGATGGTTCAG
 ACCCAGGTTCTGTTCTACAAGA^AACTTTGACAAGGGTGAGTTT^CAGCGTTGCACGACTTTACCCAAATGACCC^T
 ACCTACAAATCAGTAA^GAAAAACTATGCTGATAACAT^TACTTACGGAATGTTGACTGGAGATATCCCTCA
 TTTAACATGGAATTTGAACCGTACTTCTTCAA^AAACAC^TAAGAAAGACCC^TGCACAACAAGATGCCGGTA
 AGAAAGCTCTTAA^CACAAGGATTTTCGTCAAGCTATTCAGTTTGTCTTTGACCGAGCGTCATTTCCAAGCA
 CAACTGCAGGTCAAGATGCCAAAACA^AAGCCTTACGTAACATGCTTGTCCCACCAACATTTGTGACCAT
 TGGAGAAAGTATTTTGGTT^CAGAAGTTGAAAAGGAAATGGCAA^AACTTGGT^GATGAATGGAAGACGTTA
 ACTTAGCTGATGCTCAAGATGGTTTCTATAATCCTGAAAAGCAA^AAGCTGAGTTTGCAAAAGCCAAAGAA

SEQUENCE LISTING

GCTTTAACAGCTGAAGGTGTAACCTTCCCAGTTCAATTAGATTACCCGTGTTGACCAAGCAAACGCAGCAAC
 TGFTCAGGAAGCCCAGTCTTTCAAACAATCTGTTGAAGCATCTCTTGGTAAAGAGAATGTCATTGTCAATG
 TTCTTGAAACAGAAACATCAACTCACGAAGCCCAAGGCTTCTATGCTGAGACCCAGAACAACAAGACTAC
 GATATCATTTTCATCATGGTGGGGACCAGACTATCAAGATCCACGGACCTACCTTGACATCATGAGTCCAGT
 AGGTGGTGGATCTGTTATCCAAAAACTTGAATCAAAGCAGGTCAAAAATAAGGATGTTGTGGCAGCTGCAG
 GCCTTGATACCTACCAAACCTTCTTCTTGATGAAGCAGCAGCAATACAGACGACAACGATGCGCGCTATAAA
 GCTTACGCAAAAGCACAAGCCTACCTTACAGATAATGCCGTAGATATCCAGTTGTGGCATTGGGTGGCAC
 TCCACGAGTTACTAAAGCCGTTCCATTTAGCGGGGGCTTCTCTTGGGCAGGGTCTAAAGGTCCTCTAGCAT
 ATAAAGGAATGAACTTCAAGACAAACCTGTACAGTAAACAATACGAAAAAGCAAAAGAAAAATGGATG
 AAAGCAAAGGCTAAGTCAAATGCAAAATATGCTGAGAAGTTAGCTGATCACGTTGAAAA

SEQ ID NO: 98 amino acid sequence comprising an N-terminal leader sequence of GAS 45
 VTFMKKSKWLAAVSVAILSVSALAA

SEQ ID NO: 99 amino acid sequence comprising a fragment of GAS 45 where the N-terminal
 leader sequence is removed

CGKNASGGSEATKYKYVFNPKSLDYILTNGGGTTDVIQTQMV DGLLENDEYGNLVP SLAKDWK VSKDG
 LTYTYTLR DGVSWY TADGEEYAPV TAE D FVTGLKHAVDDKSDALYVVEDS IKNL KAYQNGEVDFKEVGVKA
 LDDKTVQYTLNKPESYWN SKT TYSVLF PVNAKFLKSKGKDFGTTDPSSILVNGAYFLSAFTSKSSMEFHKN
 ENYWDAKNVGIESVKL TYS DGS DPGSFYK NFDKGEFSVARLYPN DPTYS AKKNYADNITYGMLTGDIRHL
 TWNLNRTSFKNFKKDP AQDAGKKALNNDKFRQAIQFAFDRASFQAQTAGQDAKTALRNMLVPPFTVTTIG
 ESDFGSEVEKEMAKLGD EWKDVNLADAQDGFYNPEKAKAEFAKAKEALTAEGVTFPVQLDYPVDQANAATV
 QEAQSFKQSV EASLGKENVIVNVLETETSTHEAQGFYAETPEQQDYDISSWWGPDYQDPRTYLDIMSPVG
 GGSVIQKLG IKAGQNKDVVAAAGLDTYQTLLEAAAITDDNDARYKAYAKAQAYLTDNAVDIPVVALGGTP
 RVTKAVPFSGGFSWAGSKGPLAYKGMKLDKPVTVKQYEKAKWKAKAKAKSNAKYAEKLADHVEK

SEQ ID NO: 100 amino acid sequence comprising GAS 95

MKIGKKIVLMFTAIVLTTVLALGVYLT SAYTFSTGELSKTFKDFSTSSNKSDAIKQTRAFSILLMGVDTGS
 SERASKWEGNSDSMILVTVPNPKTKTMTSLERD LTTLSGPKNNEMNGVEAKLNAAYAAGGAQMAIMTVQ
 DLLNITIDNYVQINMQGLIDL VNAVGGITVTNEFD FPI SIAENEPEYQATVAPGTHKINGEQALVYARMRY
 DPEGDYGRQKRQREVIQKVLKILALDSISSYRKILSAVSSNMQTNIEISSRTIPSLLG YRDALRTIKTY
 QLKGEDATLSDGGSYQIVT SNHLEIQNRIRTELGLHKVNQLKTNATVYENLYGSTKSQTVNNNYDSSGQA
 PSYSDSHSSYANYSSGVDTGQSASTDQDSTASSHRPATPSSSSDALAADESSSSSGSGLVPPANINPQT

SEQ ID NO: 101 polynucleotide sequence encoding GAS 95

ATGAAAATGGAAAAAATAAGTTTTAATGTTACAGCTATTGTGTTAACTGCTTTGGCATTAGGTGT
CTATCTAACTAGTGCTTATACCTTCTCAACAGGAGAATTATCAAAGACCTTTAAAGATTTTTCGACATCTT
 CAAACAAAAGTGATGCCATTAAACAAACAAGAGCTTTTCTATCTTGTGATGGGTGTTGATACAGGCTCT
 TCAGAGCGTGCC TCCAAGTGGGAAGGAAACAGTGATTCGATGATTTTGGTTACGGTTAATCCAAAGACCAA
 GAAAACAAC TATGACTAGTTTAGAACGAGATACCTTAACCACGTTATCTGGACCCAAAAATAATGAAATGA
 ATGGTGTGTAAGCTAAGCTTAACGCTGCTTATGCAGCAGGTGGCGCTCAGATGGCTATTATGACCGTGCAA
 GATCTTTTGAATATCACCATTGATAACTATGTTCAAATTAATATGCAAGGCCTTATTGATCTTGTGAATGC
 AGTTGGAGGGATTACAGTTACAAATGAGTTTGTATTTCC TATCTCGATTGCTGAAAACGAACCTGAATATC
 AAGCTACTGTTGCGCCTGGAACACACAAAATTAACGGTGAACAAGCTTTGGTTTATGCTCGTATGCGTTAT
 GATGATCCTGAGGGAGATTATGGTCGACAAAAGCGTCAACGTGAAGTCATTCAAAGGATTTGAAAAAAT
 CCTTGCTCTTGATAGCATTAGCTCTTATCGGAAGATTTTATCTGCTGTAAGTAGTAATATGCAAACGAATA
 TCGAAATCTCTTCTCGCACTATCCCTAGTCTATTAGGTTATCGTGACGCACTTAGAACTATTAAGACTTAT
 CAACTAAAAGGAGAAGATGCCACTTTATCAGATGGTGGATCATAACAAATTTGTTACCTCTAATCATTTGTT
 AGAAATCCAAAATCGTATCCGAACAGAAATTAGGACTTCATAAGGTTAATCAATTA AAAACAAATGCTACTG
 TTTATGAAAATTTGTATGGGTCAACTAAGTCTCAGACAGTAAACAACAAC TATGACTCTTCAGGCCAGGCT
 CCATCTTATCTGATAGTCATAGCTCTTACGCTAATTAATCAAGTGGAGTAGATACCGGCCAGAGTGCTAG
 TACAGACCAGGACTCTACTGCTTCAAGCCATAGGCCAGCTACGCCGCTCTTCTTCATCAGATGCTTTAGCAG
 CTGATGAGTCTAGCTCATCAGGGTCTGGATCATTAGTTCCCTCTGCTAATATCAACCCCTCAGACCTAA

SEQ ID NO: 102 amino acid sequence comprising N-terminal leader sequence of GAS 95
 MKIGKKIVLMFTAIVLTTVLALGVYLT SAYTF S

SEQUENCE LISTING

SEQ ID NO: 103 amino acid sequence comprising a fragment of GAS 95 where the N-terminal leader sequence is removed.

TGELSKTFKDFSTSSNKSDAIKQTRAFSILLMGVDTGSSERASKWEGNSDSMILVTVNPKTKKTTMTSLER
 DTLTTLSSGPKNNEMNGVEAKLNAAAYAAGGAQMAIMTVQDLLNITIDNYVQINMQGLIDLVNAVGGITVTVNE
 FDFPISIAENEPEYQATVAPGTHKINGEQALVYARMRYDDPEGDYGRQKRQREVIQKVLKILALDSSISSY
 RKILSAVSSNMQTNIEISSRTIPSLGYYRDLALRTIKTYQLKGEDATLSDGGSYQIVTSNHLLEIQNRIRTE
 LGLHKVNQLKTNATVYENLYGSTKSQTVNNNYDSSGQAPSYSDSHSSYANYSSGVDTGQSASTDQDSTASS
 HRPATPSSSSDALAADESSSSSGSGLVPPANINPQT

SEQ ID NO: 104 amino acid sequence comprising GAS 193

MKKRKLAVTLLSTILLNSAVPLVVADTSLRNSTSSDQPTTADTDTTDESETPKKDKKSKETASQHDQK
 DHKPSHTHTPTPPSNDTKQTDQASSEATDKPNKDKNDTKQPDSSDQSTPSPKDQSSQKESQNKDGRPTSPD
 QQKQDTPDKTPEKSADKTPEKGPBKATDKTPEPNRDAPKPIQPPLAAAPVFI PWRESKDL SKLKPSSRSS
 AAYVRHWGTGSAYTHNLLSRRYGITAEQLDGLNSLGIHYDKERLNGKRLLEWEKLTGLDVRVAI VAIAMAE
 SSLGTQGVAKKGANMFYGAFFDFNPNAKKYSDEVAIRHMVEDTIIANKNQTFERQDLKAKKWSLGLQDLD
 LIDGGVYFTDTS GSGQRRADIMTKLDQWIDDHGSTPEIPEHLKITSGTQFSEVPVGYKRSQPQNVLTYKSE
 TYSFGQCTWYAYNRVKELGYQVDRYMGNGGDWQRKPGFVTT HKPKVGYVVSFAPGQAGADATYGHVAVVEQ
 IKEDGSILISESNVMGLGTISYRTFTAEQASLLTYVVGDKLPRP

SEQ ID NO: 105 polynucleotide sequence encoding GAS 193

ATGAAGAAAAGGAAATTGTTAGCAGTAACACTATTAAGTACCATACTCTTAAACAGTGCAGTGCCATTAGT
 TGTGCTGATACCTCCTTGCGTAATAGCACATCATCCACTGATCAGCCTACTACAGCAGATACTGATACGG
 ATGACGAGAGCTGAAACACCAAAAAAGACAAAAAAGCAAGGAAACAGCGTTCGACGACGACACCCAAAAA
 GACCATAAGCCTACACACTCACCAACCCCTTCAAATGATACTAAGCAGACCGATCAGGCATCATC
 TGAAGCTACTGACAAAACCAAATAAAGACAAAAACGACACCAAGCAACCAGACAGTGCATCAATCCACC
 CATCTCCCAAAGACCAGTCGTCTCAAAAAAGAGTCACAAAACAAAGACGGCCGACCTACCCCATCACCCTGAT
 CAGCAAAAAGATCAGACACCTGATAAAAACACCAGAAAAATCAGCTGATAAAAACCCCTGAAAAAGGACCAGA
 AAAAGCAACTGATAAAAACACCAGAGCCAAATCGTGACGCTCCAAAACCCATCCAACCTCCTTTAGCAGCTG
 CTCCTGTCTTTTATACCTTGGAGAGAAAGTGACAAAGACCTGAGCAAGCTAAAACCAAGCAGTCGCTCATCA
 GCGGCTTACGTGAGACACTGGACAGGTGACTCTGCCCTACACTCACAACCTGTTGTTCACGCCGTTATGGGAT
 TACTGCTGAACAGCTAGATGGTTTTTTGAAACAGTCTAGGTATTCACTATGATAAAGAAGCCTTAAACGGAA
 AGCGTTTTATTAGAATGGGAAAAACTAACAGGACTAGACGTTTCGAGCTATCGTAGCTATTGCAATGGCAGAA
 AGCTCCTAGGTACTCAGGGAGTTGCTAAAGAAAAAGGAGCCAAATATGTTTGGTTATGGCGCCTTTGACTT
 CAACCCAAACAATGCCAAAAATACAGCGATGAGGTTGCTATTCGTACATGGGTAGAAGACACCATCATTG
 CCAACAAAAACCAACCTTTGAAAGACAAGACCTCAAAGCAAAAAAATGGTCACTAGGCCAGTTGGATACC
 TTGATTTGATGGTGGGGTTTACTTTACAGATACAAGTGGCAGTGGGCAAAGACGAGCAGATATCATGACCAA
 ACTAGACCAATGGATAGATGATCATGGAAGCACACCTGAGATTCCAGAACATCTCAAGATAACTTCCGGGA
 CACAATTTAGCGAAGTGCCCGTAGGTTATAAAGAAAGTCAGCCACAAAACGTTTTGACCTACAAGTCAGAG
 ACCTACAGCTTTGGCCAATGCACCTGGTACGCCTATAATCGTGTCAAAGAGCTAGGTTATCAAGTCGACAG
 GTACATGGGTAACGGTGGCGACTGGCAGCGCAAGCCAGGTTTTGTGACCACCATAAAACCTAAAGTGGGCT
 ATGTCGCTCATTTGCACCAGGCCAAGCAGGAGCAGATGCAACCTATGGTCACGTTGCTGTTGTAGAGCAA
 ATCAAAGAAGATGGTTCATCTTAATTTAGAGTCAAATGTTATGGGACTAGGCACCATTTCCATATCGGAC
 GTTACACAGCTGAGCAGGCTAGTTTTGTTGACCTATGTCGTAGGGGACAAACTCCCAAGACCATAA

SEQ ID NO: 106 amino acid sequence comprising GAS 137

MSDKHINLVIVTGMMSGAGKTVAIQSFEDLGYFTIDNMPALVLPKFLELIEQTNENRRVALVVDMRSRLFFK
 EINSTLDSIESNPSIDFRILFLDATDGELVSRYKETRSHPLAADGRVLDGIRLERELLSPLKSMSQHVVD
 TTKLTPRQLRKTISDQFSEGSNQASFRIEVMSFGFKYGLPLDADLVFDVRF L PNPYYQVELREKTGLDEDV
 FNYVMSPHESEVFYKHLNLLIVPILPAYQKEGKSVLTVAI GCTGGQHRSVAF AHCLAESLATDWSVNESH
 R DQNRKETFVNR

SEQ ID NO: 107 polynucleotide sequence encoding GAS 137

ATGTCAGACAAACACATTAATTTAGTTATTGTGACAGGAATGAGCGCGCTGGAAAAACAGTTGCCATTCA
 GTCTTTTGGAGATCTAGGCTACTTTACCATTGATAAATATGCCCCAGCCTTGGTTCCAAAATTTTTAGAAT
 TAATTTGAACAAACCAATGAAAATCGTAGGGTGGCTTTGGTTGTCGATATGAGAAGTCGTTTGTGTTTTCAAG

SEQUENCE LISTING

GAAATTAATTCTACCTTAGATAGTATTGAAAGCAATCCTAGCATTGATTTTCGGATTCTTTTTTGGATGC
AACGGATGGAGAATTGGTGTACGCTATAAAGAAACCAGACGGAGCCACCCTTTGGCTGCGGACGGTTCGTG
TGCTTGATGGTATTCGATTGGAAAGAGAATCCTATCTCCTTTGAAAAGCATGAGCCAACATGTGGTGGAT
ACAACAAAATTGACCCCTAGACAATTGCGTAAAACCATTTAGACCAGTTTTCGAAGGGTCTAATCAAGC
CTCTTTCCGTATTGAAGTGTGAGCTTTGGGTTCAAATATGGTCTTCTTTGGATGCGGATTTGGTTTTTG
ATGTGCGTTTTCTACCCAATCCTTATTATCAGGTAGAGCTTCGTGAAAAAACAGGACTAGATGAGGACGTT
TTTAATTATGTGATGTCACCCAGAATCAGAGGTGTTTTACAAGCATTTGTTAAACCTTATTGTCCCTAT
CTTACC GGCTTACC AAAAGAAGGGAAGTCTGTCTTGACGGTGGCTATTGGCTGCACAGGAGGCCAACACC
GCAGCGTTGCCCTTGGCCATTGCTTGGCAGAAAGTCTGGCAACAGATTGGTCGGTTAATGAAAGCCATCGT
GATCAAAAATCGTCGTAAGGAAACGGTGAATCGTTCATGA

SEQ ID NO: 108 amino acid sequence comprising GAS 84

MI IKKRTVAILLAIASSFFLVACQATKSLKSGDAWGVYQKQKSI TVGF DNTFVPMGYKDESGRCKGFDIDL
KEVVFHQYGLKVN FQAINWDMKEAELNNGKIDVIWNGYSITKERQDKVAF TDSYMRNEQI IIVVKKRSDIKTI
SDMKHKVLGAQSASSGYDSLRLTPKLLKDFIKNKDANQYETFTQAFIDLKSDRIDGILIDKVYANYLLAKE
GQLENYRMIPTTFENEAF SVGLRKEDKTLQAKINRAFRVLYQNGKFQAISEKWF GDDVATANIKS

SEQ ID NO: 109 polynucleotide sequence encoding GAS 84

ATGATTATAAAAAAAGAACCCTAGCAATTTTAGCCATAGCTAGTAGCTTTTTCTTGGTAGCTTGTCAAGC
TACTAAAAGTCTTAAATCAGGAGATGCTTGGGGAGTTTACCAAAGCAAAAAGTATTACAGTTGGTTTTG
ACAATACGTTTGTTCCTATGGGCTATAAGGATGAAAGCGGCAGATGCAAAGGTTTTGATATTGATTTGGCT
AAAGAAGTTTTTACCAATATGGACTCAAGGTTAACTTTCAAGCTATTAATTGGGACATGAAAGAAGCAGA
ACTAAACAATGGTAAAATTGATGTAATCTGGAATGGTTATTCATAACTAAGGAGCGTCAGGATAAGGTTG
CCTTTACTGATCTTACATGAGAAATGAACAAATTATTGTTGTCAAAAAAGATCTGATATTAACAATA
TCAGATATGAAACATAAAGTGT TAGGAGCACAAATCAGCTTCATCAGGTTATGACTCCTTGTAAAGAACTC
TAAACTGCTGAAAGATTTTATTA AAAATAAAGACGCTAATCAATATGAAACCTTTACACAAGCTTTTATTG
ATTTAAAATCAGATCGTATCGATGGAATATTGATTGACAAAAGTATATGCCAATTACTATTTAGCAAAAAGAA
GGGCAATTAGAGAATTATCGGATGATCCCAACGACCTTTGAAAATGAAGCATTTCGGTTGGACTTAGAAA
AGAAGACAAAACGTTGCAAGCAAAAATTAATCGTGCTTTCAGGGTGCCTTTATCAAATGGCAAATTTCAAG
CTATTTCTGAGAAATGGTTTTGGAGATGATGTTGCCACTGCCAATATTAATCTTAA

SEQ ID NO: 110 amino acid sequence comprising N-terminal leader sequence of GAS 84

MI IKKRTVAILLAIASSFFLVA

SEQ ID NO: 111 amino acid sequence comprising a fragment of GAS 84 where the N-terminal leader sequence is removed

CQATKSLKSGDAWGVYQKQKSI TVGF DNTFVPMGYKDESGRCKGFDIDLAKEVVFHQYGLKVN FQAINWDMK
EAELNNGKIDVIWNGYSITKERQDKVAF TDSYMRNEQI IIVVKKRSDIKTISDMKHKVLGAQSASSGYDSL
RTPKLLKDFIKNKDANQYETFTQAFIDLKSDRIDGILIDKVYANYLLAKEGQLENYRMIPTTFENEAF SVG
LRKEDKTLQAKINRAFRVLYQNGKFQAISEKWF GDDVATANIKS

SEQ ID NO: 112 amino acid sequence comprising GAS 384

MKTLAFDTSNKTL SLAILDDETL LADMTLNIQKKHSVSLMPAIDFLMTCTDLKPDLERIVVAKGPGSYTG
LRVAVATAKTLAYSLNIALVGISSLYALAASTCKQYPNTLVVPLIDARRONAYVGYRQKSVMPQAHASL
EVIIEQLVEEQLIFVGETAPFAEKIQKLPQAILLPTLPSAYECGLLQSLAPENVDAFVPQYLKRVEAE
ENWLKDNEIKDDSHYVKRI

SEQ ID NO: 113 polynucleotide sequence encoding GAS 384

ATGAAGACACTTGCATTTGATACCTCAAATAAAAACCTTGTCCTTGGCTATACTTGTGATGATGAGACACTTCT
AGCAGATATGACCCTTAACATTCAGAAAAACATAGTGTAGCCTTATGCCTGCTATTGATTTTTTGGATGA
CTTGTACTGATCTTAAACCTCAAGATTTAGAAAGAATAGTGGTTGCAAAGGCCCTGGATCTTACACAGGT
TTACGAGTGGCAGTTGCTACTGCAAAAACCTTAGCCTACAGTTTAAATATTGCATTGGTCGGGATTTTCGAG
TCTATATGCTTTGGCTGCGTCTACTTGTAAACAGTATCCAAATACTTTGGTGGTGCCATTGATTGATGCTA
GAAGGCAAAATGCGTATGTAGGTTATTATCGGCAAGGAAAATCAGTGATGCCACAAGCCCATGCTTCACTA
GAAGTTATTATAGAACAATTAGTAGAAGAAGGACAGCTGATTTTTGTTGGGGAGACTGCTCCTTTTGTCTGA

SEQUENCE LISTING

GAAAATTCAAAAGAACTACCTCAGGCGATACTACTTCCAACCTTCCTTCTGCTTACGAATGTGGTCTTT
TGGGGCAAAGTTTGGCACCAGAAAATGTAGACGCCTTTGTCCCTCAATATCTCAAGAGAGTGGAAAGCTGAA
GAAAACCTGGCTCAAAGATAATGAGATAAAAAGATGATAGTCACTACGTTAAGCGAATCTAA

SEQ ID NO: 114 amino acid sequence comprising GAS 202

MLKRLWLILGPLLIAFVLVITIFSFPTQLDHSIAQEKANAVAITDSSFKNGLIKRQALSDETCRFVPPFFG
SSEWSRMDSMHPSVLAERYKRSYRPFLLIGKRGSAASLSHYGIQQITNEMQKKKAI FVVSPQWFTAQGINPS
AVQMYLSNTQVIEFLLKARTDKESQFAAKRLLLELNPVSKSNLLKKVSKGKSLSRDLRAILKQHQVALRE
ESLSFSLGKSTNYEKRIILPRVKGLPKVFSYKQLNALATKRGQLAT'TNNRFGIKNTFYRKRIAPKYNLYKNF
QVNYSYLASPEYNDFQLLLSEFAKRKTDVLFVITPVNKAWADY TGLNQDKYQAAVRKIKFQLKSQGFHRIA
DFSKDGGESYFMQDTIHLGWNGWLA FDKKVQPFL ETKQVPVNYKMNPFYFSKIWANRKLQ

SEQ ID NO: 115 polynucleotide sequence encoding GAS 202

ATGCTTAAGAGACTCTGGTTAATCTTAGGTCCTCTTCTTATTGCCCTTTGTTTTAGTAGTGATTACTATTTT
TAGTTTTCTACACAACCTTGATCATTCCATAGCTCAGGAAAAAGCAAATGCCGTTGCGATCACAGATAGTT
CTTTTAAAAATGGTTTGATTAAGACAAGCTTTATCAGATGAGACTTGTCGTTTTGTGCCTTTTTTTGGT
TCTAGCGAATGGAGTCGAATGGATAGTATGCACCCTTCGGTGC'TTGCAGAGCGCTACAAGCGGAGCTATAG
ACCATTTTTAATTTGGTAAGAGAGGATCAGCATCTTTGTGCGATTATTATGGTATAACAACAAATTACCAATG
AAATGCAAAAAGAAAAAGCCATCTTTGTAGTATCTCCTCAATGGTTTACTGCTCAAGGGATTAATCCTAGT
GCGGTT CAGATGTACTTGTCTAACACTCAAGTGATTGAATTTTTACTAAAAGCTAGA ACTGATAAAGAATC
ACAGTTTGCAGCAAAGCGTTTGTCTTGAGCTTAACCTGGTGTGTCTAAATCAAACCTTATTGAAAAAAGTAA
GTAAGGGTAAGTCTCTTAGTTCGGTTAGACAGAGCTATTTTGAAATGTCAACATCAAGTAGCATTGAGAGAA
GAGTCCCTTTTTAGTTTTTTAGGCAAATCTACTA ACTATGAAAAAGAATTTTGCCTCGCGTTAAGGGATT
ACCTAAAGTATTTTCGTATAACAATTTGAATGCATTAGCAACTAAGAGAGGCCAATTAGCAACAACAACA
ACCGTTTTGGGATTA AAAAATACATTTTTATCGTAAACGAATAGCACCTTAAATACAATCTTTATAAGAATTTTC
CAAGTTAATTTATAGTTTACC'TGGCGTACCAGAAATACAATGATTTTTCAGCTTTTATTATCAGAATTTGCTAA
ACGAAAAACAGATGTACTCTTTGTTATAACTCCTGTTAATAAAGCTTGGGCGGATTATAACCGCTTAAATC
AAGATAAGTATCAAGCGGCAGTTCGTAAAATAAAATTCAGTTAAAGTCACAAGGATTTTCATCGCATTGCT
GACTTCTCAAAAGATGGTGGT GAGTCTACTTTATGCAAGATACCATCCATCTCGGTTGGAATGGCTGGTT
AGCTTTTGTATAAGAAAGTGCAACCATTTCTAGAAACGAAGCAGCCAGTGCCCAACTATAAAATGAACCTT
ATTTTTATAGTAAAAATTTGGGCAAATAGGAAAGACTTGCAATAG

SEQ ID NO: 116 amino acid sequence comprising GAS 057

MEKKQRFSLRKYKSGTFSVLIGSVFLVMTTVAADELSTMSEPTITNHAQQQAQHLTNTLSSAESKSQDT
SQITLKTNREKEQSQDLVSEPTTTELDADTDAASMAN TGSDATQKSASLPVNTDVHDWVKTKGAWDKGYKG
QGKVVAVIDTGDIDPAHQSMRISDVSTAKVKSKE DMLARQKAAGINYG SWINDKVVFAHNYVENS DN IENQ
FEDFDEDWENFEFDAEAEPKAIKKHKIYRPQSTQAPKETVIKTEETDGS HDIDWTQTD DDTKYESHGMHVT
GIVAGNSKEAAAATGERFLGIAPEAQVMFMRV FANDIMGSAESLF IKAIEDAVALGADV INLSLGTANGAQL
SGSKPLMEAEI EKAKKAGVSVVVAAGNERVY GSDHDDPLATNPDYGLV GSPSTGRTPPTSVAAINSKWVIQRL
MTVKELENRADLNHGKAIYSESVDFKDIKDSLGYDKSHQFAYVKESTDAGYNAQDVKGKIALIERDPNKTY
DEMIALAKKHGALGVLIFNNKPGQSNRSMRLTANGMGI PSAFISHEFGKAMS QLNNGTGSLEFDSVVS KA
PSQKGNEMNHFSNWGLTSDGYLKPDI TAPGGDIYSTYNDNHYSQTGTSMAS PQIAGASLLVKQYLEKTQP
NLPKEKIADIVKNLLMSNAQIHVNPETKTTTSPRQQAGLLNIDGAVTSGLYVTGKDNYGSI SLGNITDTM
TFDVTVHNL SNKDKTLRYDTELLTDHVD PDKGRFTLTS HSLKTYQGGEVTV PANGKVTVRVTMDV SQFTKE
LTKQMPNGYYLEGFVRF RDSQDDQLNRVNI PFVGFKGQFENLAVAEESIYRLKSQGKTGFYFDESGPKDDI
YVGKHFTGLVTLGSETNVSTKTI SDNGLHTLGT FKNADGKFILEKNAQGNPVLAI SPNGDNNQDFAAFKGV
FLRKYQGLKASVYHASDK EHKNP LWVSPESFKGDKNFNSDIRFAKSTLLGTAFSGKSLTGAELPDGHYHY
VVSYPDVVGA KRQEMTFDMILDRQKPVLSQATFDPE TNRFKPEPLKDRGLAGVRKDSVFYLERKDNKPYT
VTINDSYKYVSVEDNKTFVERQADGSFILPLDKAKL GDFYMYMEDFAGNVAIAKLG DHPQLTGLKTPIKLK
LTDGNYQTKETLKDNL EMTQSDTGLVTNQAQLAVVHRNQ PPSQLTKMNQDFFISP NEDGNKDFVAFKGLKN
NVYNDLTVNVYAKDDHQKQTP IWSQAGASVSAIESTAWYGITARGSKVMPGDYQYVVYTRDEHGKEHQKQ
YTI SVNDKKPMTQGRFDPINGVDHFTPDKTKALDSSGIVREBEVFYLA KKNRKFDFVTEGKDGITVSNKV
YI PKNPDGSYTISKRDGVTLSDYYLVEDRAGNVS FATLRDLKAVGKDKAVVNFGLDL PVPEDKQIVNFTY
LVRDADGKPIENLEYMNSGNSLILPYGKYTV ELLTYDTNAAKLES DKIVSF TLSADNNFQOQVTFKITMLA
TSQITAHFDHLLPEGSRVSLKTAQDQLI PLEQSLYVPKAYGKTVQEGTYEVVVS LPKGYRIEGNTKVN TLP

SEQUENCE LISTING

NEVHEL^SLRLV^KVG^DAS^DST^GDK^VMS^KN^NS^QAL^TAS^AT^PTK^ST^SA^KAL^PST^GE^KM^LKL^RIV^LGL^VLL^L
GL^TCV^FSR^KK^STK^D

SEQ ID NO: 117 polynucleotide sequence encoding GAS 057

GTGGAGAAAAAGCAACGTTTTTCCCTTAGAAAAATACAAAATCAGGAACGTTTTTCGGTCTTAATAGGAAAGCGT
TTTCTTGGTGATGACAACAACAGTAGCAGCAGATGAGCTAAGCACAATGAGCGAACCAACAATCACGAATC
ACGCTCAACAACAAGCGCAACATCTCACCAATACAGAGTTGAGCTCAGCTGAATCAAAATCTCAAGACACA
TCACAAATCACTCTCAAGACAAATCGTGAAAAAGAGCAATCACAAGATCTAGTCTCTGAGCCAACCACAAC
TGAGCTAGCTGACACAGATGCAGCATCAATGGCTAATACAGGTTCTGATGCGACTCAAAAAAGCGCTTCTT
TACCGCCAGTCAATACAGATGTTACGATTGGGTAAAAACCAGGAGCTTGGGACAAGGGATACAAAAGGA
CAAGGCAAGGTTGTCGCAGTTATTGACACAGGGATCGATCCGGCCCATCAAAGCATGCGCATCAGTGATGT
ATCAACTGCTAAAGTAAAATCAAAGAAGACATGCTAGCACGCCAAAAGCCGCCGGTATTAATTATGGGA
GTTGGATAAATGATAAAGTTGTTTTTGACATAATTATGTGGAAAATAGCGATAATATCAAAGAAAATCAA
TTCGAGGATTTTGATGAGGACTGGGAAAACCTTTGAGTTTGATGCAGAGGCAGAGCCAAAAGCCATCAAAAA
ACACAAGATCTATCGTCCCCAATCAACCCAGGCACCGAAAAGAACTGTTATCAAAAACAGAAGAAACAGATG
GTTACATGATATTGACTGGACACAAAACAGACGATGACACCAAAATACGAGTCACACGGTATGCATGTGACA
GGTATTGTAGCGGTAATAGCAAAGAAGCCGCTGCTACTGGAGAACGCTTTTTAGGAATTGCACCAGAGCC
CCAAGTCATGTTTCATGCGTGTTTTTGCCAACGCATCATGGGATCAGCTGAATCACTCTTTATCAAAGCTA
TCGAAGATGCCGTGGCTTTAGGAGCAGATGTGATCAACCTGAGTCTTGGAACCGCTAATGGGGCACAGCTT
AGTGGCAGCAAGCCTCTAATGGAAGCAATTGAAAAAGCTAAAAAGCCGGTGTATCAGTTGTTGTAGCAGC
AGGAAATGAGCGCGTCTATGGATCTGACCATGATGATCCATTGGCGACAAATCCAGACTATGGTTTGGTCCG
GTTCTCCC'TCAACAGGTGCAACACCAACATCAGTGGCAGCTATAAACAGTAAGTGGGTGATTC AACGTCTA
ATGACGGTCAAAGAATTAGAAAACCGTGCCGATTTAAACCATGGTAAAGCCATCTATTCAAGAGTCTGTGCA
CTTTAAAGATATAAAGATAGCCTAGGTTATGATAAATCGCATCAATTTGCTTATGTCAAAGATCAACTG
ATGCGGGTTATAACGCACAAGACGTTAAAGGTAAAATGCTTTAAT'TGAACGTGATCCCAATAAAACCTAT
GACGAAATGATTGCTTTGGCTAAGAAAACATGGAGCTCTGGGAGTACTTATTTTTAATAACAAGCCTGGTCA
ATCAAACCGCTCAATGCGTCTAACAGCTAATGGGATGGGGATACCATCTGCTTTCATATCGCACGAATTTG
GTAAGGCCATGTCCCAATTAATGGCAATGGTACAGGAAGTTTAGAGTTTGACAGTGTGGTCTCAAAAAGCA
CCGAGTCAAAAAGGCAATGAAATGAATCATTTTCAAAT'TGGGGCC'AACTTCTGATGGCTATTTAAAACC
TGACATTACTGCACCAGGTGGCGATATCTATTCTACCTATAACGATAACCCTATGGTAGCCAAAACAGGAA
CAAGTATGGCCTCTCCTCAGATTGCTGGCGCCAGCCTTTTGGTCAAACAATACCTAGAAAAGACTCAGCCA
AACTTGCCAAAAGAAAAAATGCTGATATCGTTAAGAACC'TATTGATGAGCAATGCTCAAATTCATGTTAA
TCCAGAGACAAAACGACCACCTCACCCGCTCAGCAAGGGGCAGGATTACTTAATATTGACGGAGCTGTCA
CTAGCGGCC'TTTATGTGACAGGAAAAGACAAC'TATGGCAGTATATCATTAGGCAACATCACAGATACGATG
ACGTTTGATGTGACTGTTTACAACCTAAGCAATAAAGACAAAACAT'TACGTTATGACACAGAATTGCTAAC
AGATCATGTAGACCCACAAAAGGGCCGCTTCACTTTGACTTCTCAC'TCCTTAAAAACGTACCAAGGAGGAG
AAGTTACAGTCCCAGCCAATGGAAAAGTGACTGTAAGGGTTACCATGGATGTCTCACAGTTTCAAAAAGAG
CTAACAAAACAGATGCCAAATGGTTACTATCTAGAAGGTTTGTCCGCTTTAGAGATAGTCAAGATGACCA
ACTAAAATAGAGTAAAACATTCCTTTTGTGGTTT'TAAAAGGGCAATTTGAAAAC'TTAGCAGTTGCAGAAAGAT
CCATTTACAGATTAANAATCTCAAGGCAAAACTGGT'TTTACTTTGATGAATCAGGTCCAAAAGACGATATC
TATGTCGGTAAACACTTTACAGGACTTGTCACTCTTGGTTCAGAGACCAATGTGTCAACCAAAAACGATTTT
TGACAAATGGTCTACACACACTTGGCACCTTTAAAAATGCAGATGGCAAATTTATCTTAGAAAAAATGCC
AAGGAAACCC'TGTCTTAGCCATTTCTCCAATGGTGACAACAACCAAGATTTTGCAGCCTTCAAAGGTGTT
TTCTTGAGAAAATATCAAGGCTTAAAAGCAAGTGTCTACCATGCTAGTGACAAGGAACACAAAATCCACT
GTGGGTCAGCCAGAAAGCTTTAAAGGAGATAAAAAC'TTAAATAGTGACATTAGATTTGCAAAAATCAACGA
CCCTGTTAGGCACAGCATTTTCTGGAAAATCGTTAACAGGAGCTGAATTACCAGATGGGCATTATCATPAT
GTGGTGTCTTATTACCAGATGTGGTCCGTGCCAAACGTCAAGAAATGACATTTGACATGATTTTAGACCC
ACAAAACCGGTACTATCAAGCAACATTTGATCCTGAAAACAACCGATTCAAACCAGAACCCCTAAAAG
ACCGTGATTAGCTGGTGTTCGCAAGACAGTGTCTTTTATCTAGAAAGAAAAGACAACAAGCCCTTATACA
GTTACGATAAAACGATAGCTACAAATATGTCTAGTAGAAGACAATAAAACATTTGTGGAGCGACAAGCTGA
TGGCAGCTTTATCTTGGCCGCTTGATAAAGCAAAATTAGGGGATTTCTATTACATGGTTCGAGGATTTTGCAG
GGAACGTGGCCATCGCTAAGTTAGGAGATCACTTACCACAAAACATTAGGTAAAACACCAATTAAACTTAAAG
CTTACAGACGGTAATTATCAGACCAAAGAAACGCTTAAAAGATAATCTTGAAATGACACAGTCTGACACAGG
TCTAGTCACAAATCAAGCCAGCTAGCAGTGGTGCACCGCAATCAGCCGCAAAGCCAGCTAACAAAGATGA
ATCAGGATTTCTTTATCTCACCAAACGAAGATGGGAATAAAGACTTTGTGGCCTTTAAAGGCTTGAAAAAT
AACGTGTATAATGACTTAACGGTTAACGTATACGCTAAAAGATGACCACCAAAAACAAACCCCTATCTGGTC

SEQUENCE LISTING

TAGTCAAGCAGGCGCTAGTGTATCCGCTATTGAAAGTACAGCCTGGTATGGCATAACAGCCCCGAGGAAGCA
 AGGTGATGCCAGGTGATTATCAGTATGTTGTGACCTATCGTGACGAACATGGTAAAGAACATCAAAGCAG
 TACACCATATCTGTGAATGACAAAAACCAATGATCACTCAGGGACGTTTTGATAACCATTAATGGCGTTGA
 CCACTTTACTCCTGACAAGACAAAAGCCCTTGACTCATCAGGCATTTGTCGCGAAGAAGTCTTTACTTGG
 CCAAGAAAAATGGCCGTAATTTGATGTGACAGAAGGTAAAGATGGTATCACAGTTAGTGACAATAAGGTG
 TATATCCCTAAAAATCCAGATGGTTCTTACACCATTCAAAAAGAGATGGTGTACACTGTCAAGATTATTA
 CTACCTTGTCGAAGATAGAGCTGGTAATGTGTCTTTGCTACCTTGCGTGACCTAAAAGCGGTGCGAAAAG
 ACAAAGCAGTAGTCAATTTTGGATTAGACTTACCGGTCCCTGAAGACAAAATAAGTGAACCTTACCTAC
 CTTGTGCGGGATGCAGATGGTAAACCGATTGAAAACCTAGAGTATTATAATAACTCAGGTAACAGTCTTAT
 CTTGCCATACGGCAAATACACGGTCGAATTTGTTGACCTATGACACCAATGCAGCCAAACTAGAGTCAGATA
 AAATCGTTTCCTTTACCTTGTGAGCTGATAACAACCTCCAACAAGTTACCTTTAAGATAACGATGTTAGCA
 ACTTCTCAAATAACTGCCCACTTTGATCATCTTTTGCAGAAAGGCAGTCGCGTTAGCCTTAAAACAGCTCA
 AGATCAGCTAATCCCGCTTGAACAGTCTTTGATGTGCTTAAAGCTTATGGCAAAACCGTTCAAGAAGGCA
 CTTACGAAGTTGTTGTGACGCTGCCTAAAGGCTACCGTATCGAAGGCAACACAAAGGTGAATACCCATACCA
 AATGAAGTGCACGAATATCATTACGCCTTGTCAAAGTAGGAGATGCCCTCAGATTCACTGATCATCAA
 GGTATGTCAAAAAATAATTCACAGGCTTTGACAGCCTTGCCACACCAACCAAGTCAACGACCTCAGCAA
 CAGCAAAAAGCCCTACCATCAACGGGTGAAAAAATGGGTCTCAAGTTGCGCATAGTAGGTCTTGTGTTACTC
 GGACTTACTTGCCTCTTTAGCCGAAAAAATCAACCAAGATTGA

SEQ ID NO: 118 amino acid sequence comprising N-terminal leader sequence of GAS 57
 MEKKQRFSLRKYKSGTFSVLIGSVFLVMTTVA

SEQ ID NO: 119 amino acid sequence comprising a fragment of GAS 57 where the N-terminal
 leader sequence is removed

ADELSTMSEPTITNHAQQQAQHLTNTLSSAESKSDTSSQITLTKTNREKEQSQDLVSEPTTTELADTDAAS
 MANTGSDATQKSASLPPVNTDVHDWVKTKGAWDKYKQGKVVAVIDTGDIDPAHQSMRISDVSTAKVKSKE
 DMLARQKAAGINYGSWINDKVVFAHNYVENS DN I KENQ F E D F E D W E N F E F D A E A E P K A I K K H K I Y R P Q S T
 QAPKETVIKTEETDGS HDIDWTQTD DDTKYESHGMHVTGIVAGNSKEAAATGERFLGLIAPEAQVMFMRVFA
 NDIMGSAESLFIKAI EDAVALGADV INLSLGTANGAQLSGSKPLMEAI EKAKKAGVSVVVAAGNERVYGS
 HDDPLATNP DYGLV GSPSTGRTPTSVAAINSKWVIQRLMTVKEL ENRADLNHGKAIYSESVDFKDIKDSL
 YDKSHQFAYVKEST DAGYNAQDVKGKIALIERDPNKTYDEMIALAKKHGALGVLI FNNKPGQS NRSMRLTA
 NGMGI P S A F I S H E F G K A M S Q L N G N G T G S L E F D S V V S K A P S Q K G N E M N H F S N W G L T S D G Y L K P D I T A P G G D I
 Y S T Y N D N H Y G S Q T G T S M A S P Q I A G A S L L V K Q Y L E K T Q P N L P K E K I A D I V K N L L M S N A Q I H V N P E T K T T T S P
 R Q Q G A G L L N I D G A V T S G L Y V T G K D N Y G S I S L G N I T D T M T F D V T V H N L S N K D K T L R Y D T E L L T D H V D P Q K R
 F T L T S H S L K T Y Q G E V T V P A N G K V T V R V T M D V S Q F T K E L T K Q M P N G Y Y L E G F V R F R D S Q D D Q L N R V N I P F V
 G F K G Q F E N L A V A E E S I Y R L K S Q G K T G F Y F D E S G P K D D I Y V G K H F T G L V T L G S E T N V S T K T I S D N G L H T L G T
 F K N A D G K F I L E K N A Q G N P V L A I S P N G D N N Q D F A A F K G V F L R K Y Q G L K A S V Y H A S D K E H K N P L W V S P E S F K G
 D K N F N S D I R F A K S T T L L G T A F S G K S L T G A E L P D G H Y H V V S Y P D V V G A K R Q E M T F D M I L D R Q K P V L S Q A T
 F D P E T N R F K P E P L K D R G L A G V R K D S V F Y L E R K D N K P Y T V T I N D S Y K Y V S V E D N K T F V E R Q A D G S F I L P L D K
 A K L G D F Y Y M V E D F A G N V A I A K L G D H L P Q T L G K T P I K L K L T D G N Y Q T K E T L K D N L E M T Q S D T G L V T N Q A Q L A
 V V H R N Q P Q S Q L T K M N Q D F F I S P N E D G N K D F V A F K G L K N N V Y N D L T V N V Y A K D D H Q K Q T P I W S S Q A G A S V S A
 I E S T A W Y G I T A R G S K V M P G D Y Q Y V V T Y R D E H G K E H Q K Q Y T I S V N D K K P M I T Q G R F D T I N G V D H F T P D K T K A
 L D S S G I V R E E V F Y L A K K N G R K F D V T E G K D G I T V S D N K V Y I P K N P D G S Y T I S K R D G V T L S D Y Y Y L V E D R A G N
 V S F A T L R D L K A V G K D K A V V N F G L D L P V P E D K Q I V N F T Y L V R D A D G K P I E N L E Y N N S G N S L I L P Y G K Y T V E
 L L T Y D T N A A K L E S D K I V S F T L S A D N N F Q Q V T F K I T M L A T S Q I T A H F D H L L P E G S R V S L K T A Q D Q L I P L E Q S
 L Y V P K A Y G K T V Q E G T Y E V V V S L P K G Y R I E G N T K V N T L P N E V H E L S L R L V K V G D A S D S T G D H K V M S K N N S Q A
 L T A S A T P T K S T T S A T A K A L P S T G E K M G L K L R I V G L V L L G L T C V F S R K K S T K D

SEQ ID NO: 120 amino acid sequence comprising C-terminal hydrophobic region
 LPSTGEKMGLKLRIVGLVLLGLTCVFSRKKSTKD

SEQ ID NO: 121 amino acid sequence comprising a fragment of GAS 57 where the C-terminal
 hydrophobic region is removed

MEKKQRFSLRKYKSGTFSVLIGSVFLVMTTVADELSTMSEPTITNHAQQQAQHLTNTLSSAESKSDT
 SQITLTKTNREKEQSQDLVSEPTTTELADTDAASMANTGSDATQKSASLPPVNTDVHDWVKTKGAWDKYK
 QGKVVAVIDTGDIDPAHQSMRISDVSTAKVKSKE DMLARQKAAGINYGSWINDKVVFAHNYVENS DN I KENQ

SEQUENCE LISTING

FEDFDEDWENFEFDAEAEPKAIKKHKIYRPQSTQAPKETVIKTEETDGSHDIDWTQTD DDTKYESHGMHVT
 GIVAGNSKEAAATGERFLGIAPEAQVMFMRVFAANDIMGSAESLFIKAIEDAVALGADV INLSLGTANGAQL
 SCSKPLMEAEIEKAKKAGVSVVVAAGNERVYVYSDHDDPLATNPDYGLVGSPTSTGRTPTS VAAINSKWVIQRL
 MTVKELENRADLNHGKAIYSESVDKDIKDSLGYDKSHQFAYVKESTDAGYNAQDVKGKIALIERDPNKTY
 DEMIALAKKHGALGVLIFNPKPGQSNRSMRLTANGMGI PSAFI SHEFGKAMSQNLNGNGTGSLEFDSVVSKA
 PSQKGNEMNHF SNWGLTSDGYLKPDI TAPGGDIYSTYNDNHYGSQTGTSMAS PQIAGA SLLVKQYLEKTQP
 NLPKEKIADIVKNLLMSNAQIHVNPETKTTTSPRQQGAGLLNIDGAVTSGLYVTGKDN YGSI SLGNITDTM
 TFDVTVHNL SNKDKTLRYDTELLTDHVDPQKGRFTLTSLSLKYQGGEVTVPANGKVTVRVTMDVSOFTKE
 LTKQMPNGYYLEGFVFRFRDSQDDQLNRVNI PFVGFKGQFENLAVAEESIYRLKSQGKT GFYFDESQPKDDI
 YVGKHF TGLVTLGSETNVSTKTI SDNGLHTLGTFFKNADGKFILEKNAQGNPVLAI SPNGDNNQDFAAFKGV
 FLRKYQGLKASVYHASDKEHKNPLWVSPESFKGDKNFNSDIRFAKSTTLLGTA FSGKSLTGAELPDGHYHY
 VVSYYPDVVGAQRQEMTFDMILDRQKPVLSQATFDPETNRFKPEPLKDRGLAGVRKDS VFYLERKDNKPYT
 VTINDSYKYVSVEDNKT FVERQADGSFILPLDKAKL GDFYVMVEDFAGNVAIAKLGDHL PQT LGKTP IKLK
 LTDGNYQTKETLKNLEMTQSDTGLVTNQAQLAVVHRNQPQS QLT KMNQDFFI SPNEDGNKDFVAFKGLKN
 NVYNDLTVNVYAKDDHQKQTP IWSSQAGASVSAIESTAWYGITARGSKVMPGDYQYVV TYRDEHGKEHQKQ
 YTISVNDKPKMITQGRFDTINGVDHFTPDKTKALDSSGIVREEVFYLAKKNGRKFVDVTEGKDGITVSDNKV
 YIPKNPDGSYTI SKRDGVTLSDYVYLVEDRAGNVSFATLRDLKAVGKDKAVVNFGLDLPVPEDKQIVNFTY
 LVRDADGKPIENLEYNNSGNLSLILPYGKYTVELLTYDTNAAKLES DKIVSFTLSADNNFQQVTFKITMLA
 TSQITAHFDHLLPEGSRVSLKTAQDQLI PLEQSLYVPKAYGKTVQEGTYEVVVS LPKGYRI EGNTKVNTLP
 NEVHEL SLRLVKVGDASDSTGDHKVMSKNNSQAL TASATPPTKSTTSATAKA

SEQ ID NO: 122 amino acid sequence comprising a fragment of GAS 57 where both the N-terminal leader sequence and the C-terminal hydrophobic region are removed

ADELSTMSEPTITNHAQQQAQHLTNTLSSAESKSQDTSQITLKTNREKEQSQDLVSEPTTTELADTDAAS
 MANTGSDATQKSASLPVNTDVHDVWVKTGAWDKGYKQGVVAVIDTGIDPAHQSMRT SDVSTAKVKSKE
 DMLARQKAAGINYSWINDKVVFAHNYVENS DNIKENQFEDFDEDWENFEFDAEAEPKAIKKHKIYRPQST
 QAPKETVIKTEETDGSHDIDWTQTD DDTKYESHGMHVTGIVAGNSKEAAATGERFLGIAPEAQVMFMRVFA
 NDIMGSAESLFIKAIEDAVALGADV INLSLGTANGAQLSGSKPLMEAEIEKAKKAGVSVVVAAGNERVYVYSD
 HDDPLATNPDYGLVGSPTSTGRTPTS VAAINSKWVIQRLMTVKELENRADLNHGKAIYSESVDKDIKDSL
 YDKSHQFAYVKESTDAGYNAQDVKGKIALIERDPNKTYDEMIALAKKHGALGVLIFNPKPGQSNRSMRLTA
 NGMGI PSAFI SHEFGKAMSQNLNGNGTGSLEFDSVVS KAPSQKGNEMNHF SNWGLTSDGYLKPDI TAPGGDI
 YSTYNDNHYGSQTGTSMAS PQIAGASLLVKQYLEKTQPNLPKEKIADIVKNLLMSNAQIHVNPETKTTTSP
 RQQGAGLLNIDGAVTSGLYVTGKDNYSISLGNITDTMTFDVTVHNL SNKDKTLRYDTELLTDHVDPQKGR
 FTLTSLSLKYQGGEVTVPANGKVTVRVTMDVSOFTKELTKQMPNGYYLEGFVFRFRDSQDDQLNRVNI PFV
 GFKGQFENLAVAEESIYRLKSQGKTGFYFDESQPKDDIYVGKHF TGLVTLGSETNVSTKTI SDNGLHTLGT
 FKNADGKFILEKNAQGNPVLAI SPNGDNNQDFAAFKGVFLRKYQGLKASVYHASDKEHKNPLWVSPESFKG
 DKNFNSDIRFAKSTTLLGTA FSGKSLTGAELPDGHYHYVVSYYPDVVGAQRQEMTFDMI LDRQKPVLSQAT
 FDPETNRFKPEPLKDRGLAGVRKDS VFYLERKDNKPYT VTINDSYKYVSVEDNKT FVERQADGSFILPLDK
 AKL GDFYVMVEDFAGNVAIAKLGDHL PQT LGKTP IKLK LTDGNYQTKETLKNLEMTQS DTGLVTNQAQLA
 VVHRNQPQS QLT KMNQDFFI SPNEDGNKDFVAFKGLKN NVYNDLTVNVYAKDDHQKQTP IWSSQAGASVSA
 IESTAWYGITARGSKVMPGDYQYVV TYRDEHGKEHQKQYTI SVNDKPKMITQGRFDTINGVDHFTPDKTKA
 LDSSGIVREEVFYLAKKNGRKFVDVTEGKDGITVSDNKVYI PKNPDGSYTI SKRDGVTLSDYVYLVEDRAGN
 VSFATLRDLKAVGKDKAVVNFGLDLPVPEDKQIVNFTY LVRDADGKPIENLEYNNSGNLSLILPYGKYTVE
 LLTYDTNAAKLES DKIVSFTLSADNNFQQVTFKITMLATSQITAHFDHLLPEGSRVSLKTAQDQLI PLEQS
 LYVPKAYGKTVQEGTYEVVVS LPKGYRI EGNTKVNTLPNEVHEL SLRLVKVGDASDSTGDHKVMSKNNSQA
 LTASATPPTKSTTSATAKA

SEQ ID NO: 123 amino acid sequence of a GAS M protein

MAKNNTNRHYSRLRKLKTGTASVAVALTVLGAGFANQTEVKANGDGNPREVIEDLAANNPAIQNIRLRYENK
 DLKARLENAMVAGRDFKRAEELEKAKQALEDRKQDLEQASQDYNRANVLEKELETITREQEI NRNLLGNAKLE
 ALELAIDQASRDYHRATALEKELEEKKALELAIDQASQDYNRANVLEKELETITREQEI NRNLLGNAKLE
 LDQLSSEKEQLTIEKAKLEEEKQISDASRQSLRRDLASREAKKQVEKDLANLTAELDKVKEEKQISDASRQGLRRDLASREAKKQVEKALEEANSKLA
 QGLRRDLASREAKKQVEKDLANLTAELDKVKEEKQISDASRQGLRRDLASREAKKQVEKALEEANSKLA
 ALEKLNKELEESKLT EKEKAELOAKLEAEAKALKEQLAKQAEELAKLRAGKASDSQTPDTPKPGNKAVPGK
 GQAPQAGTKPNQNKAPMKETKRQLPSTGETANPFFTAAL TVMATAGVAAVVRKEEN

SEQUENCE LISTING

SEQ ID NO: 124 amino acid sequence of GAS SfbI

MSFDGFFLHHLTNELKENLLYGRIQKVNQPFERELVLTIRNHRKNYKLLLLSAHPVFGRVQITQADFQNPQV
 PNTFTMIMRKYLQGAIVIEQLEQIDNDRIIEIKVSNKNEIGDAIQATLI IEIMGKHSNI ILVDRAENKIIES
 IKHVGFSSQNSYRITLPGSTYIEPPKTAAVNPFTITDVPLFEILQTOELTVKSLQOHFQGLGRDTAKELAE
 LTTDKLKRFRFEPFARPTQANLTTASFAPVLFSDSHATFETLSMDLDHFYQDKAERDRINQQASDLIHRVQT
 ELDKNRNKLKQEAELLATENAELFRQKGELLTTYLSLVPNNQDSVILDNYYTGEKIEIALDKALTPNQNA
 QRYFKKYQKLKBAVKHLSGLIADTKQSITYFESVDYNLSQASIDDIEDIREELYQAGFLKSRQRDKRHKRK
 KPEQYLASDGTITILMVGRNNLQNEELTFKMAKKGELWFHAKDIPGSHV I IKDNLDPSEVKTTDAAELAAYY
 SKARLSNLVQVDMIEAKKLHKPSGAKPGFVYTYTGQKTLRVTPDQAKILSMKLS

SEQ ID NO: 125 amino acid sequence of a GAS Shp protein

MTKVVIKQLLQVIVVFMISLSTMTNLVYADKGQIYGCI IQRNRYRHPISGQIEDSGGEHSFDIGQGMVEGTV
 YSDAMLEVSDAGKIVLTFRMSLADYSGNYQFWIQPGGTGSFQAVDYNITQKGTDTNGTFLDIAISLPTVNS
 IIRGSMFVEPMGREVVFYLSASELIQKYSGNMLAQLVTEQDNSQNEVVKDSQKPVDTKLGESQDESHTGAM
 ITQNKPKANSNNKSLSDKKILPSKMGLTTSLELKKEDKFRSKKDLSIMIYFPTFFLMLGGFAVWVWKKR
 KKNDKTM

SEQ ID NO: 126 amino acids 10 to 30 of GAS protein SagA

FSIATGSGNSQGGSGSYTPGKC

SEQ ID NO: 127 polynucleotide sequence comprising fusion construct 117-40a-RR

ATGGCCTTTAACACAAGCCAGAGTGTCAAGTGCACAAGTTTATAGCAATGAAGGGTATCACCAGCATTGAC
 TGATGAAAAATCACACCTGCAATATAGTAAAGACAACGCACAACCTTCAATTGAGAAATATCCTTGACGGCT
 ACCAAAATGACCTAGGGAGACACTACTCTAGCTATTATTACTACAACCTAAGAACCCTTATGGGACTATCA
 AGTGAGCAAGACATTGAAAAACACTATGAAGAGCTTAAGAACAAGTTACATGATATGTACAATCATTATG
 tagcgggtggcggatccatgagtgtaggcgtatctcaccgaagtcagaagcagatgatagagcctcaggagaaa
 CGAAGGCGAGTAATACTCACGACGATAGTTTACCAAACAGAAAACAATTCAGAGGCAAAGGCAACTATT
 GATGCAGTTGAAAAAATCTCAGTCAACAAAAGCAGAAGTGCACAGACTTGCTACCGCTCTGACAAAAAC
 TACTGCTGAAATCAACCCTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAA
 TTTACACTAATACTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTA
 ACAGCTACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACTGCAATGTGTCAGAACA
 AAAAGCTAGCATTTTCAGCAGAACTACTCGAGCTCAAGATTTAGTGGAA CAAGTCAAAACGCTCTGAACAAA
 ATATTGCTAAGCTCAATGCTATGATTAGCAATCTGATGCTATCACTAAAGCAGCTCAAACGGCTAATGAT
 AATACAAAAGCATTAAAGCTCAGAATTGGAGAAGGCTAAAGCTGACTTAGAAAATCAAAAGCTAAAGTTAA
 AAAGCAATTGACTGAAGAGTTGGCAGCTCAGAAAAGCTGCTCTAGCAGAAAAGAGGCAGAACTTAGCTGTC
 TTAATCCTCAGCTCCGCTCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCAGGCTAT
 CCTCTTGAAGAACTTAAAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACAA
 AGAGCATGCAGATCAAATTAATGCCAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGATATTCAG
 CAGATCGTAATCGCTTTGTTGATCCCGATAATTTGACACCAGAAAGTGCAAAATGAGCTAGCGCAGTTTGCA
 GCTCACATGATTAATAGTGTACGtCgTCAATTAGGTCTACCACCAGTTACTGTTACAGCAGGATCACAAGA
 ATTTGCAAGATTACTTAGTACCAGCTATAAGAAAATCATGGTAATACAAGACCATCATTTGTCACGGAC
 AGCCAGGGGTATCAGGGCATTATGGTGTGGGCTCATGATAAACTATTAATTGAAGACTCTGCCGGAGCG
 TCAGGGCTCATTGCAAAATGATGATAACATGTACGAGAATATCGGTGCTTTAACGATGTGCATATGTGAA
 TGGTATTAACGTTGATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAATACAT
 ACGGCCATGCTATTAACCTTTTACGTGTAGATAAACATAACCTAATGC GCCTGTTTACCTTGGATTTTCA
 ACCAGCAATGTAGGATCTTTGAATGAACACTTTGTAATGTTCCAGAGTCTAACATTGCTAACCATCAACG
 CTTTAATAAGACCCCTATAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGTAGGCCTGTATCTG
 ATACTATTGCAGCGATCAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTGTCCGGCTATTCATCAAGAAGCT
 GATATTATGGCAGCCCAAGCTAAAGTAAGTCAACTTCAAGGTAAATTAGCAAGCACACTTAAGCAGTCAGA
 CAGCTTAAATCTCCAAGTGAGACAATTAATGATACTAAAGGTTCTTTGAGAACAAGAACTACTAGCAGCTA
 AAGCAAAAACAAGCACAACCTCGAAGCTACTCGTGAATCAATCATTAGCTAAGCTAGCATCGTTGAAAGCCGA
 CTGCACCAGACAGAAGCCCTTAGCAGAGCAAGCCGACAGCCAGATGACAGCACTGGTGGCTAATAAAGCTCA
 TTTGCAATATCTAAGGGACTTTAAATTTGAATCCTAACCGCTTCAAGTGATACGTGAGCGCATTGATAATA
 CTAAGCAAGATTTGGCTAAAACCTACCTCATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCT
 AAACAAAGCAGTCTAGAAGCTACTATTGCTACCACAGAACCAGTTGACTTTGCTTAAAACCTTAGCTAA
 CGAAAAGGAATATCGCCACTTAGACGAAGATATAGCTACTGTGCTGATTTGCAAGTAGCTCCACCTCTTA

SEQUENCE LISTING

CGGGCGTAAAAACCGCTATCATATAGTAAGATAGATACTACTCCGCTTGTTCAAGAAATGGTTAAAGAAACG
AAACAACACTATTAGAAGCTTCAGCAAGATTAGCTGCTGAAAATAACAAGTCTTGTAGCAGAAGCGCTTGTTGG
CCAAACCTCTGAAATGGTAGCAAGTAATGCCATTGTGTC TAAAATCACATCTTCGATTACTCAGCCCTCAT
CTAAGACATCTTATGGCTCAGGATCTTCTACAACGAGCAATCTCATTCTGATGTTGATGAAAGTACTCAA
cGtgcgggccgcaactcgagCACCACCACCACCACCAC

SEQ ID NO: 128 amino acid sequence comprising fusion construct 117-40a-RR

M A F N T S Q S V S A Q V Y S N E G Y H Q H L T D E K S H L Q Y S K D N
A Q L Q L R N I L D G Y Q N D L G R H Y S S Y Y Y Y N L R T V M G L S S
E Q D I E K H Y E E L K N K L H D M Y N H Y A S G G G S M S V G V S H Q
V K A D D R A S G E T K A S N T H D D S L P K P E T I Q E A K A T I D A
V E K T L S Q Q K A E L T E L A T A L T K T T A E I N H L K E Q Q D N E
Q K A L T S A Q E I Y T N T L A S S E E T L L A Q G A E H Q R E L T A T
E T E L H N A Q A D Q H S K E T A L S E Q K A S I S A E T T R A Q D L V
E Q V K T S E Q N I A K L N A M I S N P D A I T K A A Q T A N D N T K A
L S S E L E K A K A D L E N Q K A K V K K Q L T E E L A A Q K A A L A E
K E A E L S R L K S A P S T Q D S I V G N N T M K A P Q G Y P L E E L
K K L E A S G Y I G S A S Y N N Y Y K E H A D Q I I A K A S P G N Q L N
Q Y Q D I P A D R N R F V D P D N L T P E V Q N E L A Q F A A H M I N S
V R R Q L G L P P V T V T A G S Q E F A R L L S T S Y K K T H G N T R P
S F V Y G Q P G V S G H Y G V G P H D K T I I E D S A G A S G L I R N D
D N M Y E N I G A F N D V H T V N G I K R G I Y D S I K Y M L F T D H L
H G N T Y G H A I N F L R V D K H N P N A P V Y L G F S T S N V G S L N
E H F V M F P E S N I A N H Q R F N K T P I K A V G S T K D Y A Q R V G
T V S D T I A A I K G K V S L E N R L S A I H Q E A D I M A A Q A K V
S Q L Q Q K L A S T L K Q S D S L N L Q V R Q L N D T K G S L R T E L L
A A K A K Q A Q L E A T R D Q S L A K L A S L K A A L H Q T E A L A E Q
A A A R V T A L V A K K A H L Q Y L R D F K L N P N R L Q V I R E R I D
N T K Q D L A K T T S S L L N A Q E A L A A L Q A K Q S S L E A T I A T
T E H Q L T L L K T L A N E K E Y R H L D E D I A T V P D L Q V A P P L
T G V K P L S Y S K I D T T P L V Q E M V K E T K Q L L E A S A R L A A
E N T S L V A E A L V G Q T S E M V A S N A I V S K I T S S I T Q P S S
K T S Y G S G S S T T S N L I S D V D E S T Q R A A A L E H H H H H H

SEQ ID NO: 129 amino acid sequence comprising a linker in the 117-40a-RR construct
YASGGGS

SEQ ID NO: 130 polynucleotide sequence comprising 40a-RR-117 fusion construct

ATGAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGGCGAGTAATAC
TCACGACGATAGTTTACCAAACCAGAAACAATTCAAGAGGCAAAGGCAACTATTGATGCAGTTGAAAAAA
CTCTCAGTCAACAAAAAGCAGAAGTGCAGAGCTTGCTACCGCTCTGACAAAACTACTGCTGAAATCAAC
CACTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAATTTACACTAATACTCT
TGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCTACTGAAACAG
AGCTTCATAATGCTCAAGCAGATCAACATTCAAAAAGAGACTGCATTGTGAGAACAAAAAGCTAGCATTTCA
GCAGAACTACTCGAGCTCAAGATTTAGTGGAACAAGTCAAACCGTCTGAACAAAATATTGCTAAGCTCAA
TGCTATGATTAGCAATCCTGATGCTATCACTAAAGCAGCTCAAACGGCTAATGATAATACAAAGCATTA
GCTCAGAATTGGAGAAGGCTAAAGCTGACTTAGAAAATCAAAGCTAAAGTTAAAAAGCAATTGACTGAA
GAGTTGGCAGCTCAGAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAGTCGTCTTAAATCCTCAGCTCC
GTCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCAGCAAGGCTATCCTCTTGAAGAACTTA
AAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACAAAGAGCATGCAGATCAA
ATTATTGCCAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGATATTCAGCAGATCGTAATCGCTT
TGTTGATCCCGATAATTTGACACCAGAAGTGCAAAATGAGCTAGCGCAGTTTGCAGCTCACATGATTAATA
GTGTACGtccGtCAATTAGGTCTACCACCAGTTACTGTTACAGCAGGATCACAGAATTTGCAAGATTACTT
AGTACCAGCTATAAGAAAATCATGGTAATACAAGACCATCATTTGTCTACGGACAGCCAGGGGTATCAGG
GCATTATGGTGTGGGCCTCATGATAAACTATTATTGAA GACTCTGCCGGAGCGTCAGGGCTCATTCGAA
ATGATGATAACATGTACGAGAATATCGGTGCTTTTACGATGTGCATACTGTGAATGGTATTAAACGTGGT

SEQUENCE LISTING

ATTTATGACAGTATCAAGTATATGCTCTTTACA GATCATTTTACACGGAAATACATACGGCCATGCTATTAA
CTTTTACGTGTAGATAAACATAACCCTAATGC GCCTGTTTACCTTGGATTTTCAACCAGCAATGTAGGAT
CTTTGAATGAACACTTTGTAAATGTTTCCAGAGTCTAACATTGCTAACCATCAACGCTTTAATAAGACCCCT
ATAAAAGCCGTTTGAAGTACAAAAGATTATGCC CAAAGAGTAGGCACCTGTATCTGATACTATTGCAGCGAT
CAAAGGAAAAGTAAGCTCATTAGAAAATCGTTT GTCTGGCTATTTCATCAAGAAGCTGATATTATGGCAGCCC
AAGCTAAAGTAAGTCAACTTCAAGGTAAATTAG CAAGCACACTTAAGCAGTCAGACAGCTTAAATCTCCAA
GTGAGACAATTAATGATACATAAAGGTTCTTTG AGAACAGAATTACTAGCAGCTAAAGCAAACAAGCACA
ACTCGAAGCTACTCGTGATCAATCATTAGCTAA GCTAGCATCGTTGAAAGCCGCACTGCACCAGACAGAAG
CCTTAGCAGAGCAAGCCGAGCCAGAGTGACAG CACTGGTGGCTAAAAAGCTCATTTGCAATATCTAAGG
GACTTTAAATTTGAATCCTAACCGCTTCAAGTG ATACGTGAGCGCATTGATAATACTAAGCAAGATTTGGC
TAAAACCTACCTCATCTTTGTTAAATGCACAAGA AGCTTTAGCAGCCTTACAAGCTAAACAAAGCAGTCTAG
AAGCTACTATTGCTACCACAGAACACCAGTTGA CTTTGGCTTAAAACCTTAGCTAACGAAAAGGAATATCGC
CACTTAGACGAAGATATAGCTACTGTGCCTGAT TTGCAAGTAGCTCCACCTCTTACGGGCGTAAAACCGCT
ATCATATAGTAAGATAGATACTACTCCGCTTGT TCAAGAAATGGTTAAAGAAACGAAACAACCTATTAGAAG
CTTCAGCAAGATTAGCTGCTGAAAATACAAGTC TTGTAGCAGAAGCGCTTGTGGCCAAACCTCTGAAATG
GTAGCAAGTAATGCCATTGTGTCTAAAATCACA TCTTCGATTACTAGCCCTCATCTAAGACATCTTATGG
CTCAGGATCTTCTACAACGAGCAATCTCATTTC TGATGTTGATGAAAGTACTCAACGtgcttagcggtggcg
gatccatggcctTTAACACAAGCCAGAGTGTCA GTGCACAAGTTTATAGCAATGAAGGGTATCACCAGCAT
TTGACTGATGAAAAATCACACCTGCAATATAGT AAAGACAACGCACAACCTTCAATTGAGAAATATCCTTGA
CGGCTACCAAAATGACCTAGGGAGACACTACTC TAGCTATTATTACTACAACCTAAGAACCCTTATGGGAC
TATCAAGTGAGCAAGACATTGAAAACACTATGA AAGAGCTTAAGAACAAGTTACATGATATGTACAATCAT
TATgcggccgactcgagcACCACCACCACCAC

SEQ ID NO: 131 amino acid sequence comprising the 40a-RR-117 fusion construct

M S V G V S H Q V K A D D R A S G E T K A S N T H D D S L P K P E T I Q
E A K A T I D A V E K T L S Q Q K A E L T E L A T A L T K T T A E I N H
L K E Q Q D N E Q K A L T S A Q E I Y T N T L A S S E E T L L A Q G A E
H Q R E L T A T E T E L H N A Q A D Q H S K E T A L S E Q K A S I S A E
T T R A Q D L V E Q V K T S E Q N I A K L N A M I S N P D A I T K A A Q
T A N D N T K A L S S E L E K A K A D L E N Q K A K V K K Q L T E E L A
A Q K A A L A E K E A E L S R L K S S A P S T Q D S I V G N N T M K A P
Q G Y P L E E L K K L E A S G Y I G S A S Y N N Y Y K E H A D Q I I A K
A S A P H N Q L N Q Y R Q D I P A D R N R F V D P D N L T P E V Q N E L A Q
F A A H M I N S V R Q L G L P P V T V T A G S Q E F A R L L S T S Y K
K T H G N T R P S F V Y G Q P G V S G H Y G V G P H D K T I I E D S A G
A S G L I R N D D N M Y E N I G A F N D V H T V N G I K R G I Y D S I K
Y M L F T D H L H G N T Y G H A I N F L R V D K H N P N A P V Y L G F S
T S N V G S L N E H F V M F P E S N I A N H Q R F N K T P I K A V G S T
K D Y A Q R V G T V S D T I A A I K G K V S S L E N R L S A I H Q E A D
I M A A Q A K V S Q L Q G K L A S T L K Q S D S L N L Q V R Q L N D T K
G S L R T E L L A A K A K Q A Q L E A T R D Q S L A K L A S L K A A L H
Q T E A L A E Q A A A R V T A L V A K K A H L Q Y L R D F K L N P N R L
Q V I R E R I Q D N T K Q D L A K T T S S L L N A Q E A L A A L Q A K Q S
S L E A T I A T T E H Q L T L L K T L A N E K E Y R H L D E D I A T V P
D L Q V A P P L T G V K P L S Y S K I D T T P L V Q E M V K E T K Q L L
E A S A R L A A E N T S L V A E A L V G Q T S E M V A S N A I V S K I T
S S I T Q P S S K T S Y G S G S S T T S N L I S D V D E S T Q R A S G G
G S M A F N T S Q S V S A Q V Y S N E G Y H Q H L T D E K S H L Q Y S K
D N A Q L Q L R N I L D G Y Q N D L G R H Y S S Y Y Y Y N L R T V M G L
S S E Q D I E K H Y E E L K N K L H D M Y N H Y A A A L E H H H H H

SEQ ID NO: 132 polynucleotide sequence comprising fusion construct GAS 117 - 40a

ATGGCCCTTTAACACAAGCCAGAGTGTCAAGTGCAC AAGTTTATAGCAATGAAGGGTATCACCAGCATTGAC
TGATGAAAAATCACACCCTGCAATATAGTAAAGAC AACGCACAACCTCAATTGAGAAATATCCTTGACGGCT
ACCAAAATGACCTAGGGAGACACTACTCTAGCTA TTATTACTACAACCTAAGAACGTTATGGGACTATCA
AGTGAGCAAGACATTGAAAACACTATGAAGAGC TTAAGAACAAGTTACATGATATGTACAATCATTATgc

SEQUENCE LISTING

gaagcggtaggggataccatgagtgtaggCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAA
CGAAGGCGAGTAATACTCACGACGATAGTTTACCAAACCAGAAACAATTCAAGAGGCAAAGGCAACTATT
GATGCAGTTGAAAAAAGTCTCAGTCAACAAAAAGCAGAAGTACAGAGCTTGCTACCGCTCTGACAAAAAC
TACTGCTGAAATCAACCACATAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAA
TTTACACTAATACTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTA
ACAGCTACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGAGCTGCATTGTCAGAACA
AAAAGCTAGCATTTCAAGCAAACTACTCTGAGTCAAGATTTAGTGAACAAGTCAAACGCTGTAACAAA
ATATTGCTAAGCTCAATGCTATGATTAGCAATCCTGATGCTATCACATAAGCAGCTCAAACGGCTAATGAT
AATACAAAAGCATTAAAGCTCAGAATTGGAGAAGGCTAAAAGCTGACTTAGAAAATCAAAAAAGCTAAAGTTAA
AAAGCAATTGACTGAAGAGTTGGCAGCTCAGAAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAGTCGTC
TTAAATCCTCAGCTCCGCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCGCAAGGCTAT
CCTCTTGAAGAAGTTAAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACAA
AGAGCATGCAGATCAAATTATGCCAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGATATTCCAG
CAGATCGTAATCGCTTTGTTGATCCCGATAATTTGACACCAGAAGTGCAAAATGGCTAGCGCAGTTTGCA
GCTCACATGATTAATAGTGTAAAGAAGACAATTAGGCTTACCACCAGTTACTGTTACAGCAGGATCACAGA
ATTTGCAAGATTACTTAGTAC CAGCTATAAGAAAACCTCATGGTAATACAAGACCATTGCTACGGAC
AGCCAGGGGTATCAGGGCATTTATGGTGTGGGCTCATGATAAAAACATTATTGAAGACTCTGCCGGAGCG
TCAGGGCTCATTCGAAATGATGATAACATGTACGAGAATATCGGTGCTTTTAAACGATGTGCATACTGTGAA
TGGTATTAAACGTGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGGAAATACAT
ACGGCCATGCTATTAACTTTTTACGTGTAGATAAAACATAACCCTAATGCGCCTGTTTACCTTGGATTTTCA
ACCAGCAATGTAGGATCTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACATTGCTAACCATCAACG
CTTTAATAAGACCCCTATAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGTAGGCACTGTATCTG
ATACTATTTGCAGCGATCAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTGTGCGCTATTCATCAAGAAGCT
GATATTATGGCAGCCCAAGCTAAAGTAAGTCAACTTCAAGGTAATTAGCAAGCACACTTAAGCAGTCCAGA
CAGCTTAAATCTCCAAGTGAGACAATTAATGATACTAAAGGTTCTTTGAGAACAGAATTACTAGCAGCTA
AAGCAAAACAAGCACAACCTGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCATCGTTGAAAAGCCGCA
CTGCACCAGACAGAAGCCTTAGCAGAGCAAGCCGAGCCAGAGTGACAGCACTGGTGGCTAAAAAAGCTCA
TTTGCAATATCTAAGGGACTTTAAATTTGAATCCTAACCGCCTTCAAGTGATACGTGAGCGCATTTGATAATA
CTAAGCAAGATTTGGCTAAAACCTACCTCATCTTTGTTAAATGCACAAGAAGCTTTAGCAGCCTTACAAGCT
AAACAAAGCAGTCTAGAAGCTACTATTGCTACCACAGAACACCAGTTGACTTTGCTTAAAACCTTAGCTAA
CGAAAAGGAATATCGCCACTTAGACGAAGATATAGCTACTGTGCCGTGATTTGCAAGTAGCTCCACCTCTTA
CGGGCGTAAAACCGCTATCATATAGTAAGATAGATACTACTCCGCTTGTTCAGAAATGGTTAAAGAAACG
AAACAACTATTAGAAGCTCAGCAAGATTAGCTGTAATAACAAGTCTTTGTAGCAGAAGCGCTTGTGG
CCAAACCTCTGAAATGGTAGCAAGTAATGCCATTGTGTCTAAAATCACATCTTCGATTACTCAGCCCTCAT
CTAAGACATCTTATGGCTCAGGATCTTCTACAACGAGCAATCTCATTTCTGATGTTGATGAAAAGTACTCAA
cGtgcgccgcactcgagcACCACCACCACCACC

SEQ ID NO: 133 amino acid sequence comprising fusion construct GAS 117-40a

M A F N T S Q S V S A Q V Y S N E G Y H Q H L T D E K S H L Q Y S K D N
A Q L Q L R N I L D G Y Q N D L G R H Y S S Y Y Y Y N L R T V M G L S S
E Q D I E K H Y E E L K N K L H D M Y N H Y A S G G G S M S V G V S H Q
V K A D D R A S G E T K A S N T H D D S L P K P E T I Q E A K A T I D A
V E K T L S Q Q K A E L T E L A T A L T K T T A E I N H L K E Q Q D N E
Q K A L T S A Q E I Y T N T L A S S E E T L L A Q G A E H Q R E L T A T
E T E L H N A Q A D Q H S K E T A L S E Q K A S I S A E T T R A Q D L V
E Q V K T S E Q N I A K L N A M I S N P D A I T K A A Q T A N D N T K A
L S S E L E K A K A D L E N Q K A K V K K Q L T E E L A A Q K A A L A E
K E A E L S R L K S S A P S T Q D S I V G N N T M K A P Q G Y P L E E L
K K L E A S G Y I G S A S Y N N Y Y K E H A D Q I I A K A S P G N Q L N
Q Y Q D I P A D R N R F V D P D N L T P E V Q N G L A Q F A A H M I N S
V R R Q L G L P P V T V T A G S Q E F A R L L S T S Y K K T H G N T R P
S F V Y G Q P G V S G H Y G V G P H D K T I I E D S A G A S G L I R N D
D N M Y E N I G A F N D V H T V N G I K R G I Y D S I K Y M L F T D H L
H G N T Y G H A I N F L R V D K H N P N A P V Y L G F S T S N V G S L N
E H F V M F P E S N I A N H Q R F N K T P I K A V G S T K D Y A Q R V G
T V S D T I A A I K G K V S S L E N R L S A I H Q E A D I M A A Q A K V

SEQUENCE LISTING

S Q L Q G K L A S T L K Q S D S L N L Q V R Q L N D T K G S L R T E L L
A A K A K Q A Q L E A T R D Q S L A K L A S L K A A L H Q T E A L A E Q
A A A R V T A L V A K K A H L Q Y L R D F K L N P N R L Q V I R E R I D
N T K Q D L A K T T S S L L N A Q E A L A A L Q A K Q S S L E A T I A T
T E H Q L T L L K T L A N E K E Y R H L D E D I A T V P D L Q V A P P L
T G V K P L S Y S K I D T T P L V Q E M V K E T K Q L L E A S A R L A A
E N T S L V A E A L V G Q T S E M V A S N A I V S K I T S S I T Q P S S
K T S Y G S G S S T T S N L I S D V D E S T Q R A A A L E H H H H H H

SEQ ID NO: 134 polynucleotide sequence comprising fusion construct GAS 117-40N

ATGGCCTTTAACACAAGCCAGAGTGTCTAGTGCACAAGTTTATAGCAATGAAGGGTATCACCAGCATTTGAC
TGATGAAAAATCACACCTGCAATATAGTAAAGACAACGCACAACCTCAATTGAGAAATATCCTTGACGGCT
ACCAAAATGACCTAGGGAGACACTACTCTAGCTATTATTACTACAACCTAAGAACCGTTATGGGACTATCA
AGTGAGCAAGACATTGAAAAACACTATGAAGAGCTTAAAGAACAAGTTACATGATATGTACAATCATATG
tagcgggtgggggatacCATGAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAA
CGAAGGCGAGTAATACTCACGACGATAGTTTACCAAAACCAGAAACAATCAAGAGGCAAAGGCAACTATT
GATGCAGTTGAAAAA ACTCTCAGTCAACAAAAGCAGAACTGACAGAGCTTGCTACCGCTCTGCACAAAAA
TACTGCTGAAATCAACCACCTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAA
TTTACACTAATACTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTA
ACAGCTACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACTGCATTGTCAGAACA
AAAAGCTAGCATTTCAGCAGAAACTACTCGAGCTCAAGATTTAGTGGAACAAGTCAAACCGTCTGAACAAA
ATATGCTAAGCTCAATGCTATGATTAGCAATCCTGATGCTATCACATAAGCAGCTCAAACGGCTAATGAT
AATACAAAAGCATTAAAGCTCAGAATGGAGAAGGCTAAAGCTGACTTAGAAAATCAAAAAGCTAAAAGTTAA
AAAGCAATGACTGAAAGAGTTGGCAGCTCAGAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAGTCGTC
TTAAATCCTCAGCTCCGTCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCCGAAGGCTAT
CCTCTTGAAGAACTTAAAAAATTAGAAGCTAGTGTTATATATTGGATCAGCTAGTTACAATAATTATTACAA
AGAGCATGCAGATCAAATTATTGCCAAAGCTAGTCCAGGTAATCAATTAATCAATACCAAGcggccgcac
tcgagCACCACCACCACCACCAC

SEQ ID NO: 135

M A F N T S Q S V S A Q V Y S N E G Y H Q H L T D E K S H L Q Y S K D N
A Q L Q L R N I L D G Y Q N D L G R H Y S S Y Y Y Y N L R T V M G L S S
E Q D I E K H Y E E L K N K L H D M Y N H Y A S G G G S M S V G V S H Q
V K A D D R A S G E T K A S N T H D D S L P K P E T I Q E A K A T I D A
V E K T L S Q Q K A E L T E L A T A L T K T T A E I N H L K E Q Q D N E
Q K A L T S A Q E I Y T N T L A S S E E T L L A Q G A E H Q R E L T A T
E T E L H N A Q A D Q H S K E T A L S E Q K A S I S A E T T R A Q D L V
E Q V K T S E Q N I A K L N A M I S N P D A I T K A A Q T A N D N T K A
L S S E L E K A K A D L E N Q K A K V K K Q L T E E L A A Q K A A L A E
K E A E L S R L K S S A P S T Q D S I V G N N T M K A P Q G Y P L E E L
K K L E A S G Y I G S A S Y N N Y Y K E H A D Q I I A K A S P G N Q L N
Q Y Q A A A L E H H H H H H

SEQ ID NO: 136

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(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
14 April 2005 (14.04.2005)

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(10) International Publication Number
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- (51) International Patent Classification⁷: **A61K 39/09**
- (21) International Application Number:
PCT/US2004/024868
- (22) International Filing Date: 30 July 2004 (30.07.2004)
- (25) Filing Language: English
- (26) Publication Language: English
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60/541,565 3 February 2004 (03.02.2004) US
- (71) Applicant (for all designated States except US): **CHIRON CORPORATION** [US/US]; 4560 Horton Street, Emeryville, CA 94608-2916 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **GRANDI, Guido** [IT/US]; c/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US). **TELFORD, John** [IT/US]; C/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US). **BENSI, Giuliano** [IT/US]; C/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US).
- (74) Agents: **HALE, Rebecca, M.** et al.; Intellectual Property-R-338, Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US).
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- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 2005/032582 A3

(54) Title: IMMUNOGENIC COMPOSITIONS FOR STREPTOCOCCUS PYOGENES

(57) Abstract: The invention includes a GAS antigen, GAS 40, which is particularly suitable for use either alone or in combinations with additional GAS antigens, such as GAS 117, GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS 384, GAS 202, and GAS 057.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/024868

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/09

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, Sequence Search, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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- *Z* document member of the same patent family

Date of the actual completion of the international search

5 July 2005

Date of mailing of the international search report

23/08/2005

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Perez, C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/024868

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	-& WO 02/34771 A (CHIRON S.P.A; THE INSTITUTE FOR GENOMIC RESEARCH; TELFORD, JOHN; MASIG) 2 May 2002 (2002-05-02) claims 1-28 page 2, line 1 - page 10, line 16	1-17, 21-27
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INTERNATIONAL SEARCH REPORT

International Application No
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International Application No
PCT/US2004/024868

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WO 2004078907	A	16-09-2004	WO	2004078907 A2	16-09-2004

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Organization
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(75) Inventors/Applicants (for US only): **GRANDI, Guido** [IT/US]; c/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US). **TELFORD, John** [IT/US]; C/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US). **BENSI, Giuliano** [IT/US]; C/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

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AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, Sequence Search, WPI Data, PAJ, BIOSIS, EMBASE

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A	-& DATABASE EPO Proteins [Online] 2 February 2004 (2004-02-02), "Sequence 7466 from Patent WO0234771." XP002334316 retrieved from EBI accession no. EPOP:CQ650509 Database accession no. CQ650509 the whole document -/--	3,10-17

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Date of the actual completion of the international search 5 July 2005	Date of mailing of the international search report 28. 10. 05
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Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Perez, C
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INTERNATIONAL SEARCH REPORT

International Application No
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WO 02077183	A	03-10-2002	NONE	
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